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THE GLUTEN AND NON-GLUTEN PROTEINS[1]

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For more than a quarter of a century, and based upon the extensive researches of Osborne (1907) and his associates, the nitrogenous matter of wheat flour has been generally regarded as constituted by several distinct and well-defined individual proteins. It is customary to consider that gluten itself is essentially an intimate mixture of the two proteins, gliadin, (soluble in dilute alcohol) and glutenin, (soluble in dilute alkali) each supplementing the other in such a manner as to provide the peculiar combination of physical properties necessary to the production of leavened bread. The non-gluten protein material has been believed to consist principally of relatively small amounts, respectively, of an albumin called leucosin, and a globulin called edestin, the former being soluble in water and the latter in dilute solutions of neutral salts.

The literature records numerous investigations, including a few by the writer, in which Osborne's characterization and identification of these individual proteins have served as the foundation for the selection and use of methods for their isolation and quantitative estimation. Most of these investigations have been undertaken in the interests of gaining some insight into the fundamental causes for variations in "gluten quality," so-called.

Taken in the aggregate, and with few exceptions, these researches have produced either negative or inconclusive, and sometimes contradictory results.

It is not surprising that attempts to establish useful correlations of baking characteristics or "gluten quality" with the properties or distribution of the flour proteins have generally yielded uncertain and inconclusive results. A lack of standards for defining, measuring and recording "baking quality" itself, in terms of

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common meaning, has contributed largely to the confusion. A further complication, which this discussion is primarily intended to emphasize, is the recent accumulation of evidence which appears to establish beyond reasonable doubt the fact that Osborne's characterization of the wheat proteins has a far more limited significance than has generally been appreciated.

It is indeed difficult to draw positive conclusions or even to formulate definite opinions based upon the numerous reports of investigations dealing with the flour proteins that are scattered throughout the literature. It is possible to present volumes of evidence both for and against almost any viewpoint that one might wish to assume as to the character, identity and distribution of the components that make up the nitrogenous portion of the wheat kernel, assuming that separate and distinct components actually exist *as such* in the wheat endosperm. It is quite possible, of course, to assume that the protein of the wheat endosperm is after all nothing more nor less than a heterogeneous mass, and that the so-called "individual" proteins do not exist in the flour *as such,* but merely reflect the action of certain specified reagents upon the whole substance.

There is some available evidence in strong support of the latter viewpoint. Gortner, Hoffman and Sinclair (1929), and Hoffman and Gortner (1927) have shown that both the quantity and composition of flour protein extracted by salt solutions will vary enormously with the kind of salt, with the concentration of the salt, and with the flour itself. They are to some extent justified in their conclusion that "the salt-soluble protein fraction does not represent a mixture of albumin and globulin nor does it represent the non-gluten proteins." The method customarily used by cereal chemists for estimating the "non-gluten" or "salt-soluble" proteins in wheat flour specifies extraction with 5% K_2SO_4. This procedure is doubtless as suitable for its intended purpose as any that might be selected. It must, however, be regarded merely as an arbitrary method whereby comparative values, only, may be obtained.

Some years ago the writer was requested by the Association of Official Agricultural Chemists to serve as referee on the determination of glutenin in wheat flour. The request was granted, and work was undertaken in the belief that glutenin is indeed as well-characterized a chemical individual as would appear from Osborne's investigations. The sole and immediate object of the work as referee on glutenin was to devise an "official" method for its quantitative estimation.

Two "quantitative" methods were eventually devised and published. One has been generally designated as the Blish and Sandstedt method (1925) and the other, developed by Blish, Abbott and Platenius (1927), as the "Barium Hydroxide" method. As a criterion for the reliability of these methods, the procedure of Sharp and Gortner (1923) was used, since the latter, although indirect and laborious, must be regarded as fairly accurate and reliable if Osborne's classification and characterization is accepted. The three methods have usually been found to give reasonably concordant results when applied to normal wheat flour, although Geddes (1930) has very recently shown that such is not the case after flour has been subjected to prolonged heat treatment. The close agreement that has usually been found by various workers, where specifications were strictly followed, is doubtless due to compensating factors that at present defy explanation.

Osborne's characterization of glutenin is based upon his studies with a product extracted from wheat flour or gluten by dilute alkali, following the preliminary removal of the other proteins by salt solution and 70% alcohol, respectively. When the filtered alkaline extract is neutralized with acid, a voluminous precipitate is obtained. Osborne called this substance "glutenin" and was inclined to the belief that it represented a chemical entity of definite individuality. He apparently did not feel that the constitution of flour proteins is seriously altered by temporary solution in dilute alkali during extraction and purification.

Later studies by Blish (1916), Cross and Swain (1924), and Blish and Pinckney (1924) indicated a striking similarity, if not the actual identity, of glutenins prepared from different flours. Csonka and Jones (1927), by salting out the glutenin from alkaline extracts with ammonium sulfate, rather than precipitating by neutralization of the alkali, obtained two "fractions," coming down at different salt concentrations, and they postulated the presence in wheat of two glutelins, which they designated, respectively, as α and β glutelins.

Blish (1926) in studying the precipitation of glutenin from solution in alkaline methyl alcohol, found that the quantity precipitated varied inversely with the strength of alkali used as the dispersing and extracting agent. This, together with an observation of a noticeable disagreement among Van Slyke analyses of glutenins prepared in different laboratories, and by different workers, led Blish and Sandstedt (1929) to investigate the chemical constitution of a number of glutenin preparations in which the strength

of the dispersing alkali was the sole variable. Using the Van Slyke method for protein analysis they found consistent and very decided differences among the chemical compositions of the preparations according to variations in concentration of NaOH used for their extraction. Different lengths and degrees of exposure to alkali of the same strength will also produce glutenins of varying chemical constitution.

Blish and Sandstedt (1929) have definitely shown that "glutenin as prepared by customary methods involving extraction with or temporary solution in alkali, is a product resulting from an irreversible alteration by the action of alkali on a more complex protein body. Both yield and chemical constitution of glutenin prepared by the usual methods will vary with the concentration of alkali. It is probable that some irreversible alteration occurs when any protein material is dispersed in alkaline solution, regardless of the concentration of alkali."

Since it is now reasonably certain that "glutenin" as prepared and described by Osborne, is not a definite and well-characterized chemical individual, and since an analogous situation exists in regard to wheat "globulin," there is obviously occasion for further intensive investigation of the nature of the wheat protein or proteins in general, and of the constitution of "glutenin" in particular.

The difficulties in establishing the individuality of a protein are great, as no one has appreciated better than Osborne, himself. It is only when different and successive "fractions" of a protein show the same composition, or when a protein of definite and constant composition can be isolated by different methods that one has any basis for the assumption that he is dealing with a distinct chemical individual or group of chemical individuals. From this viewpoint gliadin is the only wheat protein that can at present lay any claim to a definite and established individuality. It shows a reasonable constancy of composition when prepared by different workers, and by various methods of isolation, although Tague (1925) found variations in *solubility* according to slight differences in methods of preparation.

Gliadin occurs in most flours to the extent of about 50% of the total protein, and its ratio to total protein is fairly constant for different flours, judging from the data of Grewe and Bailey (1927). It probably constitutes ordinarily from 55 to 60% of the total protein or crude gluten. As to the actual number, chemical identity and distribution of the other nitrogenous component or components, there is occasion for serious doubt and uncertainty. Many

individual non-gliadin protein preparations have been made in the writer's laboratory, in which the use of alkali has been avoided, and in which dilute acetic acid in alcohol has been the initial dispersing agent. From such dispersions "glutenin fractions" may be coagulated by neutralization or by the addition of salts. Omitting details here, it has been found by submitting all preparations to partial Van Slyke analysis that there is considerable variation in composition, as well as in amount, of protein precipitated according to variations among factors such as pH, temperature, time, kind of salt, amount of salt, and concentration of protein in solution. In some cases, it has been possible to isolate a "glutenin" of uniform and constant composition by different methods of procedure. One may not yet say with any certainty, however, that this is an individual protein of definite character and composition. It is certainly a protein of greater molecular size and complexity than "glutenin" as customarily prepared from alkaline extracts of flour according to the conventional procedure, and may be regarded as having undergone far less alteration during isolation. There is good evidence of the presence of at least two gluten proteins aside from gliadin. The work is being continued in the hope of eventually reaching definite and positive conclusions.

Some studies by the writer (1916) show positively that there are decided and consistent differences among flours as to the chemical constitution of their protein substance, even though these differences did not appear in the gliadins and "glutenins" prepared, respectively, from them. Some of the data then obtained may be appropriately reproduced here, for purposes of the present discussion, and these data are shown in Table I which follows.

Eight flours of widely varying origin, protein content and bak-

TABLE I.

Ammonia N After Hydrolysis of Entire Flour, as Correlated with Percentages of Total and Soluble N, Respectively

Sample No.	Total N %	Per Cent of the Total N as—	
		Water soluble (tap water)	Ammonia N after Hydrolysis
445	1.26	29.0	18.2
444	2.55	15.7	23.0
440	2.17	18.2	21.5
439	1.93	18.7	21.0
401	2.09	18.9	20.8
452	1.92	19.6	19.9
441	2.13	20.4	21.0
438	1.67	26.9	18.8

ing characteristics were used in this study. In each case a portion of the entire flour was submitted to complete acid hydrolysis. The percentages of total nitrogen liberated as ammonia N by hydrolysis varied from 18.2 to 23.00. Now the non-gluten proteins are known to yield much less ammonia N on hydrolysis than the gluten proteins. Therefore the lower values for *total* ammonia N were considered as indicating larger ratios of non-gluten protein in the flours under consideration. This idea was confirmed by estimating in each flour the percentage of total nitrogen soluble in tap water, when it was found that the percent of soluble N was inversely proportional to the total ammonia N after hydrolysis, as would be expected. The flour showing 18.2% of ammonia N after hydrolysis contained 29% soluble N, and the one showing 23% ammonia N had only 15.7% of its total N extracted by tap water. This surely indicates that flours vary considerably in their respective ratios of gluten to non-gluten protein.

It is of special interest to note that in this series of flours the samples containing the larger percentages of total protein tend to show correspondingly the smaller percentages of "soluble" or "non-gluten" protein, and vice versa. If it is in general true that flours of comparatively high total nitrogen or protein content also show a tendency to contain correspondingly greater *percentages* of that protein in the form of gluten, then the determination of total flour protein acquires added significance and importance.

Summary and Conclusions

1. Flours vary considerably, not only in amount of protein but in the composition of protein.

2. Differences in composition are probably due to variations in the distribution of the individual proteins and to the varying proportions in which they occur. The ratio of non-gluten to gluten protein appears to be the chief basis of variation.

3. Of the several individual proteins occuring in wheat flour, gliadin is the only one whose chemical individuality has been established with any degree of certainty. Its ratio to the total protein appears to be fairly constant in most flours.

4. Aside from gliadin, there are probably several other proteins, as suggested by the work of Osborne. However, there is strong evidence for the belief that these proteins have not yet been reliably identified or characterized.

5. Trustworthy and dependable methods for the identifica-

tion and quantitative separation of individual flour proteins other than gliadin are not available. It is possible to demonstrate strong tendencies toward certain *types* of chemical constitution and properties, but beyond this the evidence is uncertain and indefinite.

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STUDIES ON THE QUALITIES OF COMBINED WHEATS AS AFFECTED BY TYPE OF BIN, MOISTURE AND TEMPERATURE CONDITIONS. I

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Probably one-half the wheat in the Southwest is now harvested by means of the combine. How to store this wheat so it will keep sound and free from damage until it is milled into flour is a problem at the present time under investigation by the departments of Agricultural Engineering, Agricultural Economics, and Milling Industry at Kansas State Agricultural College. These storage experiments were made at the Fort Hays Branch Experiment Station, Hays, Kansas. Assistance was given by certain bin manufacturers and by the Hays Experiment Station. The milling, baking, and some chemical tests were made at the Department of Milling Industry. The judging of the wheat for market grade and damage was done by Mr. O. F. Phillips at the Grain Inspection Laboratory, Chicago. The tests for rancidity were made by Dr. D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C., and the germination tests were made at the seed laboratory of the Bureau of Plant Industry, Washington, D. C.

Eleven bins, each of approximately 500 bushels capacity, were erected on the grounds of the Fort Hays Station. The materials for these bins were lent by companies interested in the manufacture of grain bins. Each bin was given a number and in this report will be designated only by number. The following is a list of the bins and a description of each one:

Bin 1. Concrete stave, similar to silo construction, placed on concrete foundation and floor; outside surface waterproofed.

Bin 2. Same as No. 1 except that both outside and inside surfaces of wall were waterproofed.

Bin 3. Square, constructed of concrete boards, making a wall 5″ thick filled with horizontal air spaces; foundation and floor made of concrete.

Bin 4. Wooden bin, with lumber both inside and outside of the 2 x 6″ studs. Inside walls and roof lined with ½″ celotex boards.

Bin 5. Wooden bin similar in construction to No. 4, but with the celotex omitted.

Bins 6 and 7. Circular steel bins with ventilated side walls and

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large central flue with suction cupola on top. These bins were set on wooden floors.

Bins 8 and 9. Circular steel bins with tight side walls, metal floor and roof.

Bin 10. Circular steel bin with tight side walls, steel floor and roof, small central ventilator of steel.

Bin 11. Circular steel bin with slight ventilation in the side walls, steel floor and roof.

It should be noted that the construction of bins No. 6 and 7 afforded the most ventilation through the floors, walls and central cupola; bins No. 10 and 11 had slight ventilation, while in the others no particular provision for ventilation was made in the bin construction. In several of these bins an attempt was made to assist ventilation by means of perforated metal spouts, and spouts made of wood and screen wire.

These bins were erected in two groups. Bins 1, 2, 9, 10, and 11 constituted one group so arranged that the wheat could be unloaded from the truck and spouted to any desired bin by means of a blower elevator. This same elevator was used later in moving the wheat from one bin to another. Bins 3, 4, 5, 6, 7 and 8 made the second group so arranged that the wheat could be spouted to any bin desired by means of a tubular farm elevator, which was also used later in transferring the wheat from one bin to another, and later to the elevator.

Temperature and meteorological studies.—From three to six thermometers of the electrical resistance type were installed in each bin, except 1 and 8, and so arranged that the temperature of the wheat could be read from central points. At the beginning, temperatures were read three times a day, but later it was found that once a day was sufficient. Temperatures in the sun outside the bins were also recorded. Information on wind velocity and direction, rainfall, humidity, and air temperature was available from the office of dry land agriculture located nearby.

The meteorological data are given in Table I.

Observation of Moisture Content of Wheat as it Ripens and During Harvesting

Wheat ripe enough to cut with the harvester binder has usually a large amount of moisture. Some wheat was windrowed on June 25 when about as green as any one would ordinarily cut with a binder. A hand-threshed sample of this wheat contained 36%

moisture. The next day a similarly threshed sample gave 23.8%, while a larger sample threshed by an experimental threshing machine gave 28%. On June 29 this wheat appeared thoroughly dry, and the moisture content was 11.2%. This was very much shriveled. Wheat from another field had 28% moisture when windrowed. In the afternoon of the next day it had 21.2%. In another field which was being windrowed on June 26, the moisture content was 20.3%.

TABLE I

METEOROLOGICAL DATA FORT HAYS EXPERIMENT STATION DURING PERIOD OF WHEAT STORAGE EXPERIMENT

July 1 to September 30, 1929

Day of Month	Rainfall Inches Per Day			Relative Humidity Per Cent of Saturation Ave. of Three Readings			Wind Velocity Ave. Miles per Hr. at Noon			Temperature Noon Temperature in Sun at Bins Deg. C		
	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.	July	Aug.	Sept.
1				48	32	42	24	12	15	32	44	38
2				39	45	35	18	11	12	30	47	34
3		T	.09	26	52	51	10	17	23	34	32	20
4			.03	24	41	71	18	13	22	35	45	28
5			.08	49	47	67	8	14	18	33	27	25
6	.12	.32	.04	50	76	60	8	21	6	35	28	24
7		.01	.47	72	70	74	9	14	11	25	24	21
8	.12	.01	.38	72	89	69	22	12	9	25	24	26
9	.12	.47	.85	69	86	56	23	14	8	21	32	24
10		1.48		80	85	55	8	10	12	27	26	28
11	3.20	.01	T	61	62	54	17	4	7	36	32	30
12			.68	52	59	67	20	4	10	40	38	27
13				49	43	52	23	5	12	40	33	30
14				47	45	51	12	8	14	42	31	27
15				55	46	45	10	14	12	44	35	26
16				74	38	62	14	7		37	40	31
17	.13			64	39	43	9	17		35	35	21
18	1.47			69	63	63	14	12		33	36	19
19	.05		T	59	51	77	10	12		31	34	18
20				65	29	80	9	13		40	47	17
21				59	33	38	16	10		35	40	26
22		T		56	45	36	14	14		38	37	25
23	.11			69	40	48	11	14		36	39	27
24	1.79			84	39	52	10	8		24	40	28
25	.03			59	50	50	8	17		35		26
26		.07	.93	56	86	49	10	15		36	22	25
27		.47	T	51	70		14	7		37	38	26
28	T		.03	53	77		15	5		31	32	
29				61	66		10	6		37	30	
30				63	36		7	4		43	30	
31				31	42		10	15		43	36	

Under some conditions the wheat does not dry very rapidly after being windrowed. A sample taken from the field before windrowing June 27, had 22% moisture; the next morning a sample

taken from the windrow had 20.2%. Another sample after wheat had lain in the windrow two nights and one day had 19.4% moisture. When this wheat was picked up and threshed, three loads had the following moistures: 15.8, 18.0 and 14.6%, respectively.

Wheat in the windrow dries out rapidly during the day if the sun shines and there is a breeze. In a series of tests made during June 28 the following moistures were obtained: 16.4, 15.4, 13.6, 12.2 and 10.2%. The last sample was taken at 2:30 p. m.

The Harvest

The harvest began on June 28 when wheat which had been windrowed was picked up and threshed. The weather was hot and windy and, as has already been noted, the wheat dried very rapidly. For these reasons very little damp wheat was secured from that which was windrowed. Another combine was started on a field of standing grain which was still green in the low places. Some high-moisture wheat was secured from this field, but the threshing was not satisfactory. The machine would not handle both the green wheat on the low land and the ripe wheat on the higher land in a satisfactory manner. Bins 2, 9 and 11 were filled with this mixture of green and ripe wheat, which proved most difficult to store without damage.

Moving the Grain

The wheat was moved from one bin to another whenever it reached a temperature which was considered dangerous. One bin was always kept empty and in reserve for this purpose. Just what constitutes a dangerous temperature has not been definitely established. It no doubt depends on several conditions. In these experiments it was assumed that 45°C. was dangerous, and whenever the wheat reached this temperature it was moved and thus cooled. The blower elevator was the more effective in cooling, but the amount of cooling also depended on the outdoor temperature. Wheat moved on hot days was cooled very little, while on cool days a very decided cooling was effected.

Observations and Tests on the Wheat in the Different Bins

Samples were taken for moisture determinations at the time the bins were filled and whenever the wheat was moved. At the same time five to ten pound samples for quality studies were taken and placed in cloth sacks. Those taken at the time the bin was filled were buried in the wheat, and whenever the wheat was

moved these samples were transferred and again buried in the wheat. Thus these samples were subjected to as nearly as possible the same conditions as the rest of the wheat in the bin throughout the whole experiment. Also, when the wheat was moved, additional samples for quality studies were taken to represent the condition of the wheat at the time of moving. These were placed where they had free access to the outside air. These samples then represented the changes which had taken place in the wheat from the time of binning to the time of moving. The wheat in bins 1, 4, 5, 6, and 7 was not moved until the end of the storage period. In the others there were several moves, and at each moving of the wheat samples were taken. The results of the tests made on these samples have been assembled in Tables II to X.

Judging the Grain

At the end of the storage period, half-pound portions were taken from all the sack samples, placed in small cloth sacks and sent to the General Field Headquarters of the Federal Grain Supervision at Chicago. Here the samples were judged for market damage under the direction of Mr. O. F. Phillips, Chairman of the Board of Review. The wheat had been in the cloth sacks for a considerable time when the odor factor was judged. This probably had permitted some of the odors to disappear, and explains why some samples were graded sound when other tests showed that considerable fermentation had taken place. At the same time, the opportunity for the disappearance of odors had not been better than when such damaged wheat is passed over cleaning machines, and it shows how it may be possible to treat wheat which has suffered damage so that such damage will not be detected in grading.

Rancidity and Germination Tests

The samples used for judging the grain were sent to Dr. D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C. Here they were tested for rancidity, and then sent to the seed laboratory of the Bureau of Plant Industry where they were tested for viability. The figures for rancidity denote the number of milligrams of free fatty acids per gram of fat.

Observations on the Wheat in the Different Bins

Bin 1.—Concrete Stave. Waterproofed Outside. The first wheat placed in this bin had been threshed and then dried in sacks. It was dry, containing about 12% moisture. The rest of the bin

was filled with combined wheat which had about 13% moisture. The grain in this bin kept in perfect condition. At the junction of the wall and floor there was a small amount of mouldy wheat, represented by sample 14849. This was the only sample from this bin that graded damaged. Its percentage of germination was 0, the rancidity high, test weight low and baking qualities poor.

The data obtained on the wheat from bin 1 are found in Table II. The tests showed that none of the wheat had suffered any damage except as noted above.

Bin 2.—Concrete Stave. Waterproofed Inside and Out. This bin was filled July 3 with the dampest wheat received during the harvest. It was a mixture of green berries, too green to thresh properly, and very dry grain from the higher parts of the field. The average of all moisture determinations made on the wheat that went into this bin was 16.2%, and one load had 18.6% moisture. The temperature of the wheat was around 40°C. when it was put in, and it began to heat almost immediately. In five days it had reached 50°C. in one part of the bin. This wheat was moved with a blower elevator July 8 into a steel bin and then returned to No. 2. The moving cooled it to 32°C., from which point it gradually increased to 48°C. in twenty days.

The entire contents of this bin was in a critical condition, and would undoubtedly have been a total loss if left undisturbed. It was caked so hard that it would stand up vertically in the bin and had an odor resembling that of silage. The wheat within eight inches of the outside wall was cool and apparently much wetter than that nearer the center. This apparent wetness must have resulted from condensation of the respiration moisture from the wheat in the center of the bin.

This wheat was moved five more times during the summer, and occupied successively bins No. 11, 8, 3, 10 and 11 for varying lengths of time. By the six movings the moisture was reduced to an average of 14% on September 28 when it was moved to the elevator.

The data secured on the samples from bin 2 are given in Table III. The three samples taken at the time of filling and which had remained with the wheat throughout the entire period graded slightly sour odor. Unfortunately no samples were taken at the first moving. Three of the four samples taken at the second moving July 29, graded no damage, while one graded slightly sour odor. Some of the samples taken at subsequent movings graded no damage, others indeterminate, and some slightly sour odor.

All the wheat was dead with the exception of two samples, taken at the second moving, and in these the germination was very low. The rancidity figures were also high in all the samples, the highest being in the samples taken when the wheat was moved to the elevator. The least rancidity was in two of the samples taken when the wheat was moved the second time. The question has been raised whether the wheat in these sample sacks suffered more damage than the wheat in the bulk. The figures for rancidity are not so high as for the samples taken at the last moving, but the baking tests show that these samples had been damaged the most. According to the baking test every sample from this bin had suffered damage, some more than others, but those taken at the second moving had suffered the least, as would be expected.

Bin 3.—Square Concrete. This bin was filled in the bottom with some wheat which averaged 10.2% moisture, and this was followed by wheat which had nearly 15% moisture. Then some dry wheat was put in on top. The wheat was very warm when placed in the bin, and that part of the bin which contained the higher moisture wheat showed an increase for a while and then remained constant. Samples taken by probe did not reveal any dangerous conditions, but it was thought best to move the wheat on August 7. The moving cooled the wheat from 45°C. to 36°C. After moving the wheat the temperature went up beyond what it was before moving, and by August 30 the temperature was again 45°C. It was moved again September 2, and then taken to the elevator September 20.

The data obtained on the wheat in this bin are found in Table IV. Only one milling sample taken at the time the wheat was moved to the elevator graded "odor indeterminate." The moisture ranged between 14 and 15% at the time of the last moving of the wheat. The two high-moisture milling samples taken at the time of binning, which remained with the wheat the entire period, gave zero germination, and almost similar results were obtained from the two samples taken when the wheat was moved to the elevator. The highest germination was obtained in the two samples taken at the first moving.

Only two samples had undergone no fermentation as shown by the rancidity tests: one taken from the driest wheat at the time of filling, and one taken near the top at the time of the second moving. The rancidity figures are, however, as a rule, much lower than obtained on the wheat from bin No. 2. The baking tests also showed much better results. On the whole it cannot be said

that wheat in bin No. 3 had suffered any damage which would seriously lower its milling and baking value. The low germination and the fairly high rancidity in some of the samples showed that this wheat had in part suffered some deterioration. As considerable 15% moisture wheat went into this bin, and as the temperature increased to 45°C. on two occasions, some deterioration would be expected.

Bin 4.—Wooden Bin. Celotex Lined. The moisture content of the wheat which went into bin 4 ranged from 16.4 to 12.2%, most of it near the latter figure. The temperature of the wheat as it was put into the bin was near 36°C. It lowered to 34°C. the first part of August, then rose to 38°C. by September 5. When moved to the elevator it had a temperature of 33°C.

The data obtained on the samples from bin 4 are found in Table V. All the samples from this bin graded "no damage," and except for one sample, the rancidity tests were low. The baking results also did not indicate any damage. The sample having 15.4% moisture at the time of binning had zero germination, and the highest rancidity. While part of the wheat put in bin 4 had as high moisture as some of the wheat put in bin 3, the moisture content was lower in the greater portion. The other important factor contributing to the better results in bin 4 was the low initial temperature, which was considerably lower than for bin 3.

Bin 5.—Wooden Bin. Ordinary Construction. The wheat put in this bin ranged in moisture content from 11.6 to 13.2%. The temperature of the wheat at the beginning was near 45°C, but there was a steady decline in temperature of the wheat in this bin. In August it ranged from 35 to 30°, and on September 25 it had gone down to 25°C.

The data obtained on the samples from this bin are found in Table VI. All samples graded "no damage." The rancidity figures for all the samples were that of wheat which had in no way undergone fermentation. The baking tests also showed that the wheat was sound.

Bin 6.—Ventilated Steel. The first wheat harvested by the pick-up method from windrowed wheat was stored in this bin. It averaged 16% moisture, including one load which contained 18%. The maximum temperature of this wheat was about 35°C. at the beginning of the storage period, and showed a gradual decline to 25°C. and less towards the close. The fact that this wheat went in at a comparatively low temperature was to its advantage.

The data obtained on the wheat in this bin are found in Table VII. Two of the samples taken at the time of binning graded slightly sour odor. These two had 15.2 and 18% of moisture respectively when placed in the bin. These two also gave high rancidity figures and were low in baking value. The figures for germination were comparatively high except for three samples, two of which were taken when the wheat was moved to the elevator. It should be noted that the moisture content in three of the samples taken when the wheat was moved to the elevator was high, yet on the whole the wheat kept very well in this bin. No sample showed any great damage in the baking test. The samples with fairly high rancidity gave smaller loaf volumes than the rest.

Bin 7.—Ventilated Steel. The wheat placed in this bin was not quite so damp as that in bin 6. The temperature of the wheat when binned was near 35°C., but soon declined to 30°C., at which it remained until near the end of the period, when it declined to 23°C. The data obtained on the wheat in this bin are found in Table VIII. The changes occurring in this bin were even less than those in bin No. 6. All samples showed high baking qualities.

Bin 9.—Tight Walled Steel. Wheat with an average moisture content of 15.4% was placed in this bin on July 4, a very hot day. Some loads apparently had a considerable higher moisture. The temperature of the wheat was 45°C. After five days it was moved, not because the temperature showed any increase, but because the odor indicated that it was spoiling. The wheat was badly caked and smelled like silage. The moving lowered the temperature to 36°C. It was moved a total of five times during the storage period, and at each moving its temperature was lowered. The highest temperature of 49°C. was reached on July 29. After the moving on August 19, the temperature was lowered from 45°C. to 35°C. After this it rose gradually to 42°C. on September 6, when it began to fall, reaching 29°C. when the wheat was moved to the elevator on September 27.

The data obtained on the wheat in bin 9 are given in Table IX. The moisture content in bin 9 was not on the whole greater than that of the wheat placed in bins 6 and 7, but the temperature was much higher at the time of binning. Furthermore, bin 9 was not as well ventilated as bins 6 and 7. It must be admitted that the high temperature of the wheat when placed in bin 9 was a disadvantage, and the results were not altogether due to the bin construction. The wheat was not quite as damp as that put into bin 2,

but it was at somewhat higher temperature. In germination, rancidity and baking tests the wheat from bin 9 was quite similar to that from bin 2. The wheat was practically all dead, the rancidity, except one sample, very high, the baking tests except in a few cases showed poor results.

Bin 11.—Steel Bin With Slight Wall Ventilation. This bin was filled with damp wheat made up of green and ripe kernels similar to that placed in bin 2. The average moisture content was 15.6%, the highest 16.3% and the lowest 14.0%. The wheat was fairly cool: 36°C. at the time of binning (July 1). It increased slightly the next few days, after which the temperature went down. The hot weather in the middle of July seemed to start the heating, the temperature reading 48°C. on July 20 when the wheat was moved. The temperature rose steadily again, reading 49°C. on August 5 when the wheat was moved again. The temperature was lowered only 7° by this moving, but it remained fairly constant until August 18, when there was a rapid rise reaching 43°C. August 23 when the wheat was moved again. This moving cooled it to 36°C. After this there was only a slight increase and when finally moved to the elevator the temperature was 37°C.

The data obtained on the wheat in this bin are found in Table X. The wheat was dead in most of the samples, and the rancidity was high except in three samples. In these two respects the wheat was quite similar to that in bins 2 and 9, but the baking results indicated somewhat less damage, showing that the wheat in bin 11 had not suffered so much damage on the whole as that in bins 2 and 9. It had the advantage of having been binned at a somewhat lower temperature, and the bin had some wall ventilation.

Ventilation Flues

Several types of ventilation flues were tried in the bins of damp wheat as a means of preventing damage. These were placed vertically in the wheat and allowed to extend above the wheat in the bin. As far as could be observed these flues were of no value. They seemed to be more harmful than beneficial, since there was usually an area of excessively damp wheat around these ventilators. The same was true of the small central ventilators furnished with some of the steel bins. Around these small central flues there was always an area of grain wetter than anywhere else in the bin. Such damp wheat became moulded when the wheat was left undisturbed for some time. This condition of dampness was not observed with the larger central flues of bins 6 and 7.

Effectiveness of Moving Wheat

Moving wheat to prevent serious or total damage seems to be quite effective. Undoubtedly the weather conditions have a large influence upon the results obtained. If the wheat is caked, the moving breaks up this condition and permits better air circulation. Hot pockets are broken up and redistributed. Mouldy spots and damp areas in them are mixed with the drier wheat and the harmful effects minimized.

The cooling effect depends upon the outside air temperature and temperature of wheat. Based upon the movement of 16 bins of wheat, it appears that the wheat can be cooled two-thirds of the difference between the wheat temperature and the outside air temperature. The temperature of the contents of one bin moved on a hot day was lowered only two degrees.

The drying effect of moving is more difficult to measure. Moisture samples show considerable variation and since the number of samples was inadequate, an absolute record of moisture content was not secured. There seems to be no definite relation of moisture reduction to the moving of wheat which can be stated from the results obtained. The wheat was undoubtedly dried somewhat by each movement. Six movements reduced the moisture of the wheat in bin No. 2 over 2%. If wheat were moved in a completely saturated atmosphere it might take on additional moisture. But the relative humidities are comparatively low in the wheat belt during the harvest season and it seems fairly certain that any moving, especially with a blower elevator, would reduce the moisture content.

Relation of Moisture Content to Rancidity

As a rule the lower the moisture content of the wheat, the less the rancidity. High rancidity generally developed when the moisture content was 15% and above. Rancidity seems also to result from heating. For the same moisture content, not so high rancidity developed in bins 6 and 7 as in 2, 9 and 11. With high rancidity was also associated poor baking results; however, a comparatively small amount of rancidity may not injure baking qualities. Notably higher ash flour was obtained from wheat in bins 2, 9 and 11 in which the most heating took place, and where the highest rancidity was developed.

TABLE II—BIN I

West Concrete, Not Water Proofed on Inside. One Load Cut June 29, Dried in Sacks, Was Put in Bottom of Bin July 1, Rest Was Filled with Wheat Combined July 2. Wheat Dry

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14793	From wheat dried in sacks placed in the center near bottom. Appeared dry.		28.0	6.97	11.7	56.6	68.5	.56	1670	91
14794	Combined wheat, placed in center of bin. Appeared dry.	13.2	39.5	8.20	13.6	55.8	70.0	.56	1830	95
	Moved to elevator Sept. 27, 1929									
14846	Taken near top of bin. Wheat sound.	12.0	3.0	6.68	14.5	54.7	67.3	.69	1870	96
14847	Taken about center of bin. Wheat sound.	13.3	12.5	7.84	14.5	55.5	65.5	.58	1850	96
14848	Taken near bottom of bin. Wheat sound.	14.1	15.0	7.49	13.7	55.3	69.0	.62	1900	97
14849	Spoiled wheat near edge. About 90% damage. (All in Bin 1 except 14849 graded; no damage.)		0	23.80	14.5	47.3	60.9	.76	1600	80

Effectiveness of Moving Wheat

Moving wheat to prevent serious or total damage seems to be quite effective. Undoubtedly the weather conditions have a large influence upon the results obtained. If the wheat is caked, the moving breaks up this condition and permits better air circulation. Hot pockets are broken up and redistributed. Mouldy spots and damp areas in them are mixed with the drier wheat and the harmful effects minimized.

The cooling effect depends upon the outside air temperature and temperature of wheat. Based upon the movement of 16 bins of wheat, it appears that the wheat can be cooled two-thirds of the difference between the wheat temperature and the outside air temperature. The temperature of the contents of one bin moved on a hot day was lowered only two degrees.

The drying effect of moving is more difficult to measure. Moisture samples show considerable variation and since the number of samples was inadequate, an absolute record of moisture content was not secured. There seems to be no definite relation of moisture reduction to the moving of wheat which can be stated from the results obtained. The wheat was undoubtedly dried somewhat by each movement. Six movements reduced the moisture of the wheat in bin No. 2 over 2%. If wheat were moved in a completely saturated atmosphere it might take on additional moisture. But the relative humidities are comparatively low in the wheat belt during the harvest season and it seems fairly certain that any moving, especially with a blower elevator, would reduce the moisture content.

Relation of Moisture Content to Rancidity

As a rule the lower the moisture content of the wheat, the less the rancidity. High rancidity generally developed when the moisture content was 15% and above. Rancidity seems also to result from heating. For the same moisture content, not so high rancidity developed in bins 6 and 7 as in 2, 9 and 11. With high rancidity was also associated poor baking results; however, a comparatively small amount of rancidity may not injure baking qualities. Notably higher ash flour was obtained from wheat in bins 2, 9 and 11 in which the most heating took place, and where the highest rancidity was developed.

TABLE II—BIN I

West Concrete, Not Water Proofed on Inside. One Load Cut June 29, Dried in Sacks, Was Put in Bottom of Bin July 1, Rest Was Filled with Wheat Combined July 2. Wheat Dry

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14793	From wheat dried in sacks placed in the center near bottom. Appeared dry.		28.0	6.97	11.7	56.6	68.5	.56	1670	91
14794	Combined wheat, placed in center of bin. Appeared dry.	13.2	39.5	8.20	13.6	55.8	70.0	.56	1830	95
	Moved to elevator Sept. 27, 1929									
14846	Taken near top of bin. Wheat sound.	12.0	3.0	6.68	14.5	54.7	67.3	.69	1870	96
14847	Taken about center of bin. Wheat sound.	13.3	12.5	7.84	14.5	55.5	65.5	.58	1850	96
14848	Taken near bottom of bin. Wheat sound.	14.1	15.0	7.49	13.7	55.3	69.0	.62	1900	97
14849	Spoiled wheat near edge. About 90% damage. (All in Bin 1 except 14849 graded; no damage.)		0	23.80	14.5	47.3	60.9	.76	1600	80

TABLE III—BIN NO. 2

CONCRETE, NOT WATER PROOFED ON INSIDE. COMBINED JULY 3 FROM LOW LAND. WHEAT QUITE IMMATURE. AVERAGE MOISTURE OF ALL SAMPLES 16.2%; MAXIMUM MOISTURE 18.6%

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14795	Placed at center near bottom, graded slightly sour odor.	17.0	0	26.03	13.8	53.4	68.3	.71	1430	88
14796	Placed 3 feet from bottom away from ventilators. Graded slightly sour odor.	18.6	0	29.75	13.2	53.6	68.0	.69	1435	88
14797	Placed at center, near top. Graded slightly sour odor.	17.6	0	24.76	14.9	54.1	66.5	.73	1455	88
	Wheat moved July 8, no samples									
	Wheat moved July 29. Average moisture 16.2%; maximum moisture 16.7%									
14824	Taken near door, bottom. Graded no damage.	15.6	4.0	26.45	15.0	53.3	71.8	.59	1810	94
14825	Taken near top, wheat caked, graded no damage.	16.0	1.5	15.76	14.3	54.8	71.0	.61	1680	92
14826	Taken near center, wheat caked, graded no damage.	15.6	0	30.76	14.2	55.0	71.3	.62	1690	92
14827	Taken at center near bottom. Graded slightly sour.	16.7	0	14.83	13.95	54.4	67.8	.63	1620	89

	Wheat moved August 5. Average moisture 14.5%; maximum 15.2%									
14840	Taken near center at top, caked, hot. Graded no damage.	15.2	0	29.40	13.6	55.3	70.3	.63	1670	88
14841	Taken near bottom center, caked, hot. Graded odor indeterminate.	15.0	0	19.19	14.3	55.7	71.0	.63	1780	94
	Wheat moved August 13. Average moisture 14.3%; maximum 14.5%									
14836	Taken near bottom center, 47° C. Graded no damage.	14.4	0	32.36	14.0	56.2	71.3	.68	1590	90
14837	Taken near bottom center, 47° C. Odor indeterminate.	14.4	0.5	26.17	14.4	56.2	70.3	.62	1770	94
	Wheat moved August 24. Average moisture 14.3%; maximum 15.4									
14842	Top center 45° C. Graded no damage.	14.3	0	26.12	14.0	56.1	69.3	.65	1650	90
14843	Bottom center 45° C. Graded odor indeterminate.	14.4	0	34.00	14.0	55.9	72.3	.67	1590	88
	Wheat moved to elevator September 28. Average moisture 14.0%; maximum 14.2%									
14866	Near top, little musty. Graded no damage.	14.2	0	33.79	14.0	55.3	70.0	.66	1600	85
14867	Center, little musty. Graded slightly sour odor.	14.0	0	32.90	14.2	56.1	70.0	.66	1640	90
14868	Center, little musty, graded no damage.	13.9	0	32.35	14.0	55.7	71.3	.66	1540	85
14869	Bottom, little musty, graded no damage.	14.1	0	32.90	14.0	55.8	70.3	.65	1570	85

TABLE IV—BIN NO. 3

CONCRETE. WHEAT WINDROWED JUNE 26. THRESHED AND BINNED 28, 29. MOISTURE OF WHEAT AT BINNING: 10.2; 15.2; 15.0; 14.6; 12.0; 13.0; 12.0%

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14798	Placed near bottom center, graded no damage.	10.2	18.0	8.24	11.2	58.3	68.8	.58	1745	93
14799	Placed near center of bin, graded no damage.	15.2	0	15.04	14.5	51.8	68.0	.69	1780	95
14800	Placed near top center, graded no damage.	14.6	0	25.96	15.1	50.4	67.8	.76	1670	91
	Moved August 7. Average moisture 14.4%; maximum 15.3%									
14832	Taken at top center, caked, graded no damage.	14.2	29.5	15.89	15.0	50.8	66.0	.65	1780	94
14833	Taken at bottom, center caked, graded no damage.	15.3	43.5	14.56	14.0	52.4	68.7	.71	1890	94
	Moved September 2. Average moisture 14.4%; maximum 14.9%									
14834	Taken near bottom center, graded no damage.	14.2	4.0	19.21	14.9	52.2	67.3	.68	1845	92
14835	Taken near top, center caked, graded no damage.	14.8	23.5	10.83	13.9	55.5	68.3	.60	1660	90
	Moved September 20 to elevator. Average moisture 14.5%; maxmium 17.3%									
14844	Graded odor indeterminate.	14.7	0	17.77	13.5	54.1	68.7	.69	1610	90
14845	Graded no damage.	14.5	0.5	18.46	15.1	51.9	66.7	.59	1860	97

TABLE V—BIN NO. 4

Wood, Double Walls, Insulated with Celotex. Wheat Windrowed June 26, Threshed June 28.
Moistures: 16.4; 15.4; 13.6; 12.2%. Average 14.4%; Maximum 16.4

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14801	Placed at center near bottom.	15.4	0	20.16	11.0	56.5	70.5	.60	1675	93
14802	Placed at center near middle.	13.6	0.5	11.15	10.9	58.2	69.8	.58	1690	91
14803	Placed at center near top. All graded, no damage.	12.2	22.5	8.18	11.0	58.9	69.8	.58	1860	97
	Moved to elevateor September 28									
14852	Taken near top center.	13.1	34.5	8.06	11.1	58.5	68.3	.58	1580	90
14853	Taken near bottom center. Both graded no damage.	14.3	26.0	8.66	10.8	58.8	70.0	.56	1620	90

TABLE VI—BIN NO. 5

Wood, Single Walls. Wheat Combined July 4. Moistures 13.2; 11.6

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14804	Placed at center 3 feet from floor.	13.2	23.5	8.59	13.8	55.9	72.0	.58	1695	93
14805	Placed at center 6 feet from floor. Both graded no damage.	11.6	21.0	6.44	13.2	56.9	71.0	.56	1840	97
	Moved to elevator September 28									
14854	Taken near top.	12.3	13.0	6.60	13.2	57.2	70.5	.56	1900	96
14855	Taken near center. Both graded no damage.	...	28.5	7.55	13.5	56.1	70.8	.55	2035	97

TABLE VII—BIN NO. 6

STEEL, SUCTION VENTILATOR AT TOP. DRUM IN CENTER FROM TOP TO BOTTOM, PERFORATED WALLS. MAXIMUM AMOUNT OF NATURAL VENTILATION. WHEAT WINDROWED JUNE 26, ALMOST RIPE, SPOTS OF UNRIPE WHEAT. STARTED THRESHING 3:30 P. M. JUNE 27. MOISTURES: 15.8; 14.6; 18.0; 15.2%

No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14806	Check, not placed in bin, no damage.	15.8	73.0	5.40	11.5	57.9	70.8	.55	1865	97
14807	Placed near bottom 12 in. from wall, no damage.		58.5	7.23	13.3	55.7	68.8	.59	1980	96
14808	Placed near center, no damage.	14.6	24.0	5.73	11.0	59.0	70.0	.56	1680	92
14809	Placed near center toward top. Graded slightly sour odor.	18.0	23.5	14.82	11.9	55.4	69.8	.59	1645	91
14810	Placed near top. Graded slightly sour odor.	15.2	5.0	16.38	11.7	55.8	69.8	.60	1630	90
14811	Placed almost on top, no damage.		69.5	7.09	11.6	58.0	69.0	.53	1720	92
14812	Threshed from wheat having 26 per cent moisture when windrowed June 26; no damage.	10.2	31.0	6.27	12.8	54.8	68.0	.57	1840	95
	Moved to elevator September 28									
14856	Taken near top.	12.2	23.5	6.98	10.9	58.2	68.0	.56	1690	92
14857	Taken near center where 18 per cent moisture wheat was put.	16.2	2.5	16.88	11.5	56.4	70.8	.59	1710	90
14858	Near center.	15.3	19.5	12.40	11.5	57.2	70.0	.54	1690	92
14859	Taken near ventilator. All graded no damage.	16.0	6.5	13.54	11.7	55.8	69.3	.58	1780	92

TABLE VIII—BIN NO. 7

Steel Bin, Drum in Center, Perforated and Extending to the Top Which Is Connected with Suction Ventilator. Walls Perforated All Around. Wheat Combined July 2. Moistures: 16.0; 14.6; 13.8%

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14813	Placed away from ventilators near bottom.	16.0	30.5	9.60	14.7	55.0	68.0	.54	1895	98
14814	Placed near ventilator.	14.6	20.0	11.49	14.5	55.0	69.5	.55	1905	98
14815	Placed away from ventilators, all graded no damage.	13.8	21.0	8.76	14.9	56.0	69.8	.57	1850	97
	Moved to elevator September 28									
14860	Taken near ventilator.	15.5	27.5	7.60	13.5	55.6	67.5	.55	1955	97
14861	Taken near center.	14.1	16.0	7.50	14.1	55.2	68.8	.56	1930	97
14862	Taken near center. All graded no damage.	16.0	18.0	12.00	13.1	55.1	69.0	.59	1820	96

TABLE IX—BIN NO. 9

Steel Bin, no Special Ventilators. Wheat Combined July 4. Moistures: 15.2; 15.6%

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
14816	Placed at center of bin, graded no damage. One sack was prepared but not put in and one sack which was put in was lost. Odor indeterminate.	15.6	.5	10.80	15.1	55.5	68.5	.55	1850	95
	Moved July 10. No samples									
	Moved July 20. Moistures: 16.7; 16.9%. No milling samples									

TABLE IX (Continued)

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour %	Ash %	Loaf Volume cc.	Texture of Bread
	Moved July 29. Moistures: 15.8; 15.9									
14828	Taken at bottom center.	15.8	.0	23.71	14.2	55.3	70.3	.67	1555	88
14829	Taken at top center.	15.9	1.0	18.32	14.4	55.2	70.3	.62	1730	92
	Both graded slightly sour odor.									
	Moved August 6. Moistures 15.6; 15.2									
14830	Taken at top center. Graded no damage. Heating.	15.6	.5	26.40	14.2	56.0	70.5	.61	1680	91
14831	Taken at bottom center. Caked, hot. Graded slightly sour odor.	15.2	1.5	23.97	14.5	56.1	71.3	.63	1725	94
	Moved August 20. Moistures: 14.3; 14.5%									
15027	Taken at bottom center, caked, temp. 46° C.	14.3	.0	31.60	15.7	56.2	71.3	...	1705	90
15028	Taken at top center, caked, temp. 46° C.	14.5	.0	27.40	15.5	56.1	70.5	.67	1625	88
	Both graded no damage.									
	Moved to elevator September 28									
14863	Taken at top center. Graded no damage.	14.7	.0	29.86	14.4	55.7	70.3	.63	1640	89
14864	Taken at center. Graded slightly sour.	14.6	.0	30.74	14.3	56.1	69.0	.65	1580	88

TABLE X—BIN NO. 11

STEEL BIN. ONE-HALF OF BIN FILLED WITH SCREEN VENTILATORS. WHEAT COMBINED JULY 1. MOST DEAD RIPE, SPOTS SOMEWHAT GREEN. MOISTURES: 16.3; 16.0; 15.8; 16.0; 14.0; 14.8.

Serial No.	Description	Moisture %	Germination %	Rancidity	Protein %	Test Wt. at Lab.	Flour % %	Ash	Loaf Volume cc.	Texture of Bread
14817	Placed at bottom center. Graded slightly sour odor.	16.3	.0	26.76	14.7	53.9	68.5	.61	1730	94
14818	Placed at bottom center. Graded no damage.	16.0	.0	33.50	14.8	54.2	70.0	.63	1800	95
14819	Placed at center. Graded no damage.	15.8	.0	26.45	14.8	53.4	70.8	.63	1685	91
14820	Placed at center. Graded slightly sour odor.	16.0	.0	30.33	15.3	55.7	68.8	.62	1720	92
14821	Placed at center near top. Graded no damage.	14.0	8.0	10.63	14.7	55.0	69.8	.56	1835	97
14822	Placed at center near top. Graded no damage.	14.8	12.0	9.0	14.6	55.9	69.8	.57	1885	97
	Moved July 20									
14823	Moisture av. 15.8, maximum 17.4, graded no damage.		4.0	20.78	15.1	54.6	72.3	.60	1710	91
	Moved August 5									
14838	Taken from top center, hot.	14.2	.0	21.48	13.9	55.0	70.7	.66	1660	90
14839	Taken from bottom center, hot caked.	15.4	70.5	9.26	13.2	56.8	70.3	.55	1840	97
	Both graded no damage.									
	Moved August 23									
15029	Bottom center caked. Graded slightly sour odor.		.5	28.35	14.2	55.9	70.0	.62	1680	90
15030	Top center caked, graded no damage.		1.5	15.30	14.0	56.1	70.5	.57	1810	94
	Moved to elevator September 28									
14850	Taken from top.	15.1	.0	19.57	13.8	54.9	67.8	.60	1830	94
14851	Taken from bottom.	14.4	.0	15.44	13.6	55.7	67.8	.58	1635	90
	Both graded no damage.									

Variation in the Wheat

One object of this experiment was to compare different bins as to their effectiveness in storing combined wheat. For such comparison all the bins should have been filled with the same kind of wheat. In the first place the wheat was harvested by two different methods. Bins 3, 4 and 6 were filled with wheat that had been windrowed and allowed to dry in the field before threshing. Such wheat cut immature and then dried will not have the same qualities as wheat normally ripened. Immaturity at the time of cutting probably accounts for the low germination in some of the samples from bins 3, 4 and 6. In the second place the wheat varied in protein. The wheat put in bins 4 and 6 had from two to three per cent lower protein than that put in the other bins. Baking qualities are not the same in wheats differing so much in protein even if the conditions of storage are the same. In the third place the initial temperature of the wheat varied considerably. The temperatures at the time of binning were as follows: bins 2 and 3, 41°; bin 4, 37°; bin 5, 45°; bin 6, 38°; bin 7, 36°, bin 9, 45°; and bin 11, 36°C. The lower initial temperature was an advantage to the wheat in bins 6 and 7. The high temperature was a disadvantage to the wheat in bins No. 9 and 5, but in the others the wheat was so dry that no injury resulted, while in No. 9 considerable heating took place.

VITAMIN D AND THE ANTIRACHITIC ACTIVATION OF FOODS BY IRRADIATION WITH ULTRA-VIOLET LIGHT

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(Read at the Convention, May, 1930)

Neither rickets nor vitamin D is new. Rickets was, to our knowledge, first thoroughly described by a physician in the second century A. D. and it has been a common affliction of most temperate zone peoples ever since. Similarly, we can logically believe that vitamin D has been in existence as long as have plant and animal life. It is, however, only during the last ten to twenty years that it has been recognized as such.

That rickets incidence is extremely high among infants and children generally is the concensus of opinion of leading nutritional and clinical investigators. The percentage occurrence in most instances runs well up between sixty and ninety per cent. Rickets is due largely to a lack of proper calcium and phosphorus metabolism. It might therefore be caused by a dietary lack of calcium, phosphorus, or of vitamin D which aids materially in the assimilation of these two mineral elements which go to form such a large part of bone, or it might be caused by dietary imbalance of these three food constituents. Rickets not only leads to malformation of the bones and teeth but, it is now believed, also renders the victim more susceptible to other diseases. It is well to keep in mind the fact that a combination of calcium, phosphorus, vitamin D, and total food intake, together with age, rate of growth, exposure to sunshine and a multiplicity of other factors enter into any rachitic consideration.

To the Frenchman, Rosseau, we give much credit for his early recognition of the great value of body exposure to the sunshine at high altitudes as a means of preventing and alleviating infantile rickets. More recently Huldschinsky (1919-1920) first pointed out the possibilities of artificially irradiating children for rickets prevention. This direct irradiation of the body with lamps has become quite common. Another means of combating rickets is the long practiced feeding of cod-liver oil and oils of other fish. This, too, as you all know, is very effective.

The Activation of Foods

In 1923 and 1924, Dr. H. Steenbock of Wisconsin (1924a; Steenbock and Black, 1924) and Dr. A. F. Hess (1924a, 1924b;

Hess and Weinstock, 1924) of New York City independently and practically simultaneously discovered that many antirachitically inert food materials could be rendered rickets-preventing by exposure to the ultra-violet light rays of either the carbon arc or mercury vapor lamp. This discovery made it possible to accomplish a long realized need—namely, that of rendering available in a variety of foods a source of vitamin D sufficiently abundant to be of great value to the welfare of large numbers of humans.

To enable the activation process to become commercially utilizable as well as to prevent unscrupulous commercial exploitation, Dr. Steenbock patented the process. The Steenbock patent (1924b) was assigned to and is administered by the Wisconsin Alumni Research Foundation. Licenses for the use of the process have already been granted in a number of food manufacturing fields both in America and in Europe. Among the American users are six pharmaceutical houses producing irradiated ergosterol in concentrated form for human consumption, one company manufacturing irradiated breakfast foods, one dry milk company, one yeast company, two biscuit manufacturers, and two companies concerned with the utilization of the process for animal feed concentrates.

You will pardon me, I hope, if I am guilty of speaking more about the irradiation of cereal breakfast foods than about the irradiation of other food products, for I am most familiar with the treatment of these materials. To a large extent, however, what I may say of the irradiation of breakfast foods is probably true also of the irradiation of other foods. The plant operation necessary to accomplish this antirachitic activation is a matter of exposure of the substance to ultra-violet light of a relatively concentrated nature. Sunlight itself is not a sufficiently potent source of ultra-violet light to be practical for bulky food activation. The best practical sources of these light rays are the mercury vapor quartz tube lamp and the carbon arc lamp. To say that one of these types of lamps is better than the other would be to speak blindly. Only by a rigorous comparison of the two—each in its most improved modern form—could one come to a fair estimation of their respective values. In the plant the cereals are conveyed in a very thin layer on an endless belt and pass under a battery of ultra-violet lamps. The technique of this exposure must be thoroughly understood and the variable factors rigidly controlled. Among these factors one might mention the kind of lamps, the power level at which they operate, their age, number, distance from the material being irradiated and facilities for preventing dust accumulation

on their bulbs or protective screens, the kind of material being irradiated, its thickness on the belt, the production stage of the product, the facilities for turning the material to expose a maximum of surface, and perhaps even the type of soil and climatic conditions under which the raw material was grown. Other methods of exposing the food to light rays might be employed. For example, the material might be allowed to fall through a slot device in a thin film exposed to the light or it could be agitated in a container while exposed to a light. Liquids might well be sprayed through an illuminated atmosphere.

Antirachitic potency once imparted to a food material is not easily lost. It has been found to be stable under ordinary household cooking conditions as well as to storage under varying degrees of temperature (Steenbock et al, 1924) for periods of practical duration. In commercial baking, however, it is possible to destroy the antirachitic factor by not sufficiently limiting the temperature.

Chemical Nature and Determination of Activation

As I have previously mentioned, most of our common foodstuffs are antirachitally activatable. Some of the exceptions are water, sugars and other non-fatty materials. They do, however, possess this property to varying degrees depending largely on their content of ergosterol. It is this relatively rare sterol first discovered by Tanret and recently recognized by Windaus and Hess (1927) as well as by Rosenheim and Webster (1927) in this connection which is now believed to be the all-important constituent which takes the antirachitic activation. As it occurs in nature in grains, yeasts, molds, and in other plant and animal tissue it is entirely inactive. Exactly what change it undergoes when exposed to ultra-violet rays is not known but it is thought that the change is only minor in nature and on the order of a shift of the double bonds. Ergosterol is a solid, white, crystallizable alcohol. We look upon it as the mother substance of vitamin D. Thus far we know it to occur most abundantly in the lower types of plant life such as yeasts and molds. The percentage of it in other plant and animal tissue is very, very small—likely only a small part of one per cent at most. Its ability to assume a tremendous potency accounts for the fact that minute quantities of it can become of great significance. Recent determinations indicating a difference in the mode of action of irradiated ergosterol and cod-liver oil (Hess, Weinstock, and Rivkin, 1930) are not surprising in view of their great inherent differences.

There are no satisfactory quantitative chemical tests for measuring vitamin D. The antirachitic potency of irradiated foods can be determined with the use of rats by any one or more of several biological methods which are available. Chief in importance among these laboratory methods are the so-called "line test" (McCollum et al, 1922) developed at Johns Hopkins, the histological picture method of studying bone formation, the following of the fecal pH, the use of X-ray pictures of the bones, and the determination of the percentage ash of certain long bones. No one of these methods taken alone is entirely satisfactory. As compared to chemical determinations they are all very slow, requiring from six to forty days for the completion of a determination.

Plate 1. Wrists of Rats Fed Three Different Rations, Each Over a Thirty-five Day Period

Rat 124

Rat 210

Rat 131

Rat 124 received a ration of which 19% was irradiated farina in a ration such as No. 3, Table 1. Rat 210 received only non-irradiated farina in a ration such as No. 1, Table 1. Rat 131 received the stock colony ration of this laboratory. This ration entirely prevents rickets and has the following composition: yellow corn 47.00%, whole milk powder 33.33%, linseed meal 10.67%, crude casein 3.33%, butterfat 3.33%, alfalfa meal 1.33%, bone ash 0.67%, and sodium chloride 0.34%.

The bones of rat 131 are entirely normal; those of rat 124 are almost normal. Rat 210, however, shows a distinctly rachitic condition as manifested by the wide cartilagenous metaphyses of the radius and ulna.

The "line test" which is dependent upon the laying down of a calcium phosphate deposit at the provisional zone of calcification in the metaphysis of long bones is quite satisfactory for estimating the vitamin D in such concentrates as cod-liver oil and viosterol. For less concentrated sources of vitamin D it is not so adaptable.

The method based on the lowering of fecal pH has resulted in great success at the hands of Jephcott and Bacharach (1926) of England, while others have encountered various difficulties in its manipulations.

X-ray pictures give a very vivid record of perfection of bone formation but like the two proceeding methods it gives numerically comparative data only indirectly. Plate I shows the wrists of rats

131, 210, and 124. Rat 131 received the stock colony ration during the thirty-five day prophylactic experimental feeding period. Rat 210 received non-irradiated farina in a ration such as Ration 1, Table I, and rat 124 received irradiated farina at the level of 19%, comparable to Ration 3, Table I, all under otherwise similar conditions. The relative perfection of bone formation can readily be noted by a comparison of calcification in the respective wrists.

As yet, the method based on the determination of the percentage ash in the long bones (the femurs are commonly used) seems to be most acceptable for assaying of common food materials for antirachitic potency. In this test a series of rations based on either Steenbock and Black's ration 2965 (1925) or on McCollum's ration 3143 (McCollum et al, 1921) are fed to a like number of groups of rats over a definite period. Table I gives a representation of the composition of such a series of rations. This series is based on

TABLE I.
A SERIES OF RATIONS DESIGNED FOR THE PROPHYLACTIC EVALUATION OF A FOOD FOR ITS ANTIRACHITIC POTENCY

Ingredients	Ration 1 (control) %	Ration 2 %	Ration 3 %	Ration 4 %
Material "A" (untreated)	76	..	57	66.5
Material "A" (irradiated)	..	76	19	9.5
Wheat Gluten	14	14	14	14
Northwestern Yeast	6	6	6	6
Calcium Carbonate	3	3	3	3
Sodium Chloride	1	1	1	1

Steenbock's diet 2965 and differs from it only in the presence of the Northwestern Powdered Yeast Foam Tablets and the displacement of the yellow corn by "Material A". "Material A" might be any food substance which one would desire to essay for its antirachitic value. It might be, for example: corn, wheat, a breakfast food, bread, or a combination of them. Diet 2965 is particularly suitable as an experimental rachitogenic ration because of its high calcium and its low phosphorus and vitamin D contents.

The relative protection from rickets afforded by the above rations may be diagnosed from the comparative femur ash analyses of the respective groups. Approximate (although not directly numerical) comparisons can be made from X-ray pictures of the legs of rats fed as indicated above.

After these rations have been fed to the respective groups of at least four rats each for thirty-five days the rats are killed and the femurs removed and extracted in a Soxhlet extractor with alcohol for about four days. The bones are then dried and weighed, ashed, and again weighed. The percentage ash in the dried ex-

tracted bones is calculated and the comparisons made directly. Subminimal sufficient levels of vitamin D produce subnormal ash values. An ash value of fifty per cent or more might be considered normal. Depending on the severity of the rickets the percentage ash would vary downward to as low as thirty or even twenty per cent. In the irradiation of breakfast foods it has been, and is, the objective of the manufacturer to impart a degree of antirachitic potency which gives complete protection to the rat against rickets when fed at the highest level as in ration 2. Diluted levels such as represented by rations 3 and 4 do not, however, entirely prevent rickets. By following this policy there is no danger, according to good authorities in nutrition, of overdosage of vitamin D even if a person ate nothing but irradiated foods. A manufacturer who would negligently fail to set a maximum as well as a minimum standard of potency would be guilty of an injustice to the public.

Some scientists are inclined to look upon the potency of irradiated foods as being so low, relatively speaking, as to be negligible. However, irradiated foods are not intended to supplant cod-liver oil or viosterol, which are medicinal foods. In this connection, as is well known, milk and eggs are two of our best and most widely distributed naturally occuring vitamin D containing foods. The vitamin D content of eggs varies markedly, depending on the ration fed to the hens. Likewise, milk from cows exposed to the sun and on pasture is higher in vitamin D content than is the milk from cows not so treated. The potency of irradiated foods compare favorably with these two naturally occuring sources of vitamin D when they are at their best. It is not necessary, nor even desirable, that irradiated foods, or that eggs, milk, or the leafy vegetables should be comparable with cod-liver oil and viosterol in vitamin D potency. Due to the high levels at which they are consumed, they appreciably augment the antirachitic properties of the ordinary diet which in many cases includes neither cod-liver oil nor viosterol.

Summary

(1) Rickets is believed to occur to the extent of seventy to ninety per cent of our infant population and together with dental caries constitutes one of the most widely occurring physical imperfections of mankind.

(2) This situation is in large part due to the limited consumption of vitamin D, which condition is caused by its limited occurrence in our natural foods.

(3) Most foods can be antirachitically activated by exposure to ultra-violet light such as that of a mercury vapor or carbon arc lamp.

(4) Potency thus imparted is believed to be due to an intramolecular rearrangement in the ergosterol molecule.

(5) Antirachitic potency of irradiated foods is highly stable to ordinary storage and household cooking but is subject to destruction at some of the higher temperatures combined with certain other variable factors existent in commercial baking. The exact degree of stability cannot be predicted; it must be determined.

(6) With rigid biological control to insure a desirable and definitely known degree of antirachitic activation the irradiation of foods has large possibilities for improving the human diet.

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CEREALS AND MINERAL METABOLISM

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(Read at the Convention, May, 1930)

The spread between potencies of cod-liver oil and irradiated ergosterol on the one hand, and most ordinary foods except egg yolk on the other hand, is so great as to cause some to consider the latter as negligible sources of vitamin D. It is commonly thought that good sources of vitamin D are so sparsely distributed that one is inclined to forget the possible value of foods relatively poor in this vitamin but ordinarily consumed in considerable volume. Such foods might be effective at least where the diet is reasonably well balanced with respect to calcium and phosphorus; and in this connection it is to be noted that in the usual vitamin D testing procedure the test material is called upon to cure rickets of an extreme degree. Quantitative determinations of the vitamin D content of less prolific sources of this vitamin, perhaps using less severe rickets-producing basal diets more in keeping with average dietary conditions, should bring forth interesting comparisons. Sherman (1926) has strongly emphasized this viewpoint.

The extremely important place which cereals occupy in the modern diet justifies their examination with respect to the foregoing considerations. Mellanby (1925), and Green and Mellanby (1928) incline to the view that there are present in the grains in varying degree certain constituents interfering with deposition of mineral matter in bone. Sherman (1925), and Steenbock, Black and Thomas (1927) express the view that the nutritional shortcomings of these food materials are readily compensated by appropriate dietary regulation. Mellanby, likewise, noted the improvement following adjustment in calcium and vitamin D consumption. The present report deals with data offering additional illustration of these relationships.

Experimental

Two series of experiments were conducted involving the feeding to rats of rations containing 60% whole wheat and 60% oat flakes. Details of these diets are recorded in Table I. Each series contained four groups in which the rations were characterized by different calcium : phosphorus ratios. A modified Osborne-Mendel salt combination lacking calcium and phosphorus was used as the

basal salt mixture and the desired calcium: phosphorus ratios then obtained by adding appropriate amounts of calcium lactate or a phosphate mixture. The chief points of interest regarding the calcium: phosphorus ratios are that in the A groups this ratio was very high; in the B and C groups it approached unity; and in the D groups it was very low. The B and C groups have the further difference that in the former the calcium and phosphorus consumption levels were relatively low and in the latter relatively high.

Inasmuch as it was planned to conduct these observations over an extended period of time it was desired to make the diet as adequate as possible consistent with the purpose of the experiment. It was considered that a sufficiency of vitamin B would be

TABLE I.

Details of Rations

Wheat Series Oat Series	82-A 83-A	82-B 83-B	82-C 83-C	82-D 83-D
	Per cent	Per cent	Per cent	Per cent
Wheat Kernel or Oat Flake	60.0	60.0	60.0	60.0
Dried Egg White	15.0	15.0	15.0	15.0
Starch	3.0	7.9	0.0	3.7
Crisco	10.4	10.4	10.4	10.4
Butterfat	3.0	3.0	3.0	3.0
Basal Salt Mixture	2.0	2.0	2.0	2.0
Calcium Lactate, Anhydrous	6.6	1.7	6.6	1.2
Phosphate Mixture*	0.0	0.0	3.0	4.7
Ca: P Ratio**				
(Wheat Series	4.8	1.35	1.35	0.20
(Oat Series	5.2	1.51	1.38	0.21

*A mixture of HNa_2PO_4 anhydrous and $H_2NaPO_4.H_2O$ in the proportion of 6 moles of the former and 4 moles of the latter.

**Computed in part from Sherman's tabulations (1926).

provided by the 60% grain components of the diets. Vitamin A was provided by means of 3% butter fat. The use of butter fat is of course open to the criticism that it may contribute a small amount of vitamin D and thus detract from the sharpness of the results. McCollum and Simmonds (1927), however, stated that 15 to 30% of butter fat were required to obtain even faint calcification of the bones. McCollum, Simmonds, Becker and Shipley (1926) reported that rachitogenic rations containing 5% butter fat "produced an exaggerated form of rickets. Calcification of the cartilage in the tibiae of these animals was entirely wanting." During the autumn, however, these diets resulted in bones that "were almost normal in structure." Reduction of the butter fat content to 1 and 2% sufficed for the production of severe rickets.

Variations in the vitamin D of the butter fat doubtless due to differences in environment and feed can of course be a complicating factor. Inasmuch, however, as the wheat and oat experiments were conducted simultaneously and butter fat drawn from the same batch for each group of experiments, and inasmuch as pronounced rickets was evidently induced as shown by X-ray examinations, it is probable that this was not a seriously disturbing factor in these experiments. It may have operated to render the diets less severely rachitogenic, thus permitting development of differences to be presently described.

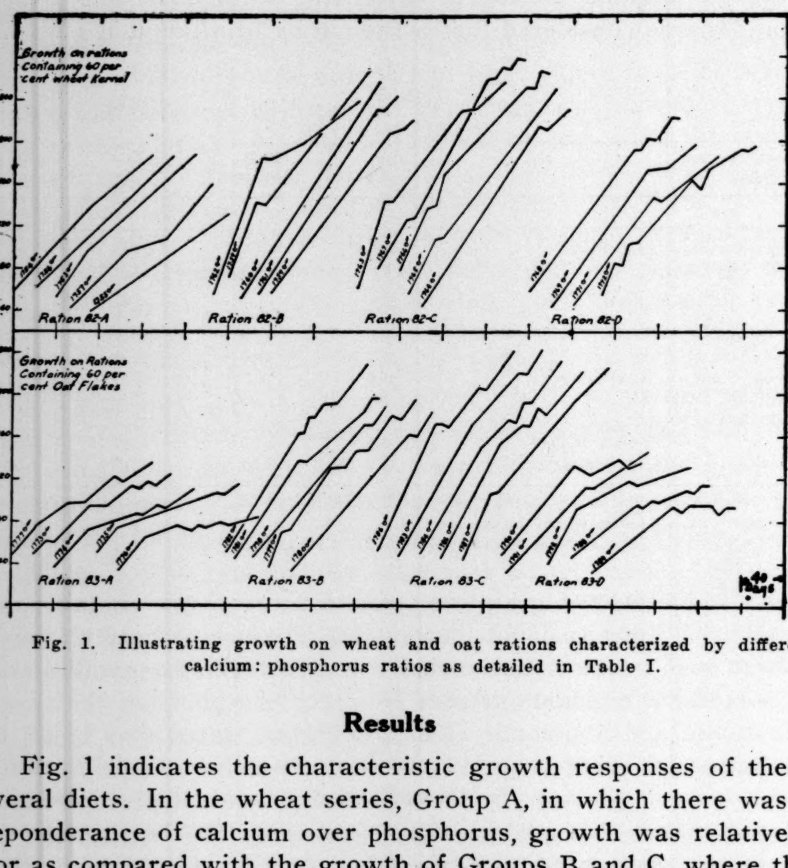

Fig. 1. Illustrating growth on wheat and oat rations characterized by different calcium: phosphorus ratios as detailed in Table I.

Results

Fig. 1 indicates the characteristic growth responses of these several diets. In the wheat series, Group A, in which there was a preponderance of calcium over phosphorus, growth was relatively poor as compared with the growth of Groups B and C, where the calcium: phosphorus ratio was fairly close to unity, regardless of whether the consumption level was high or low. In Group D, the diet of which was characterized by a preponderance of phosphorus over calcium, growth was not so good as in Groups B and C but

better than in Group A. A similar relationship is shown in the oatmeal group. One notes here, however, a distinct difference in comparison with the wheat series. Growth was in practically all cases poorer in the oat series than in the comparable wheat groups.

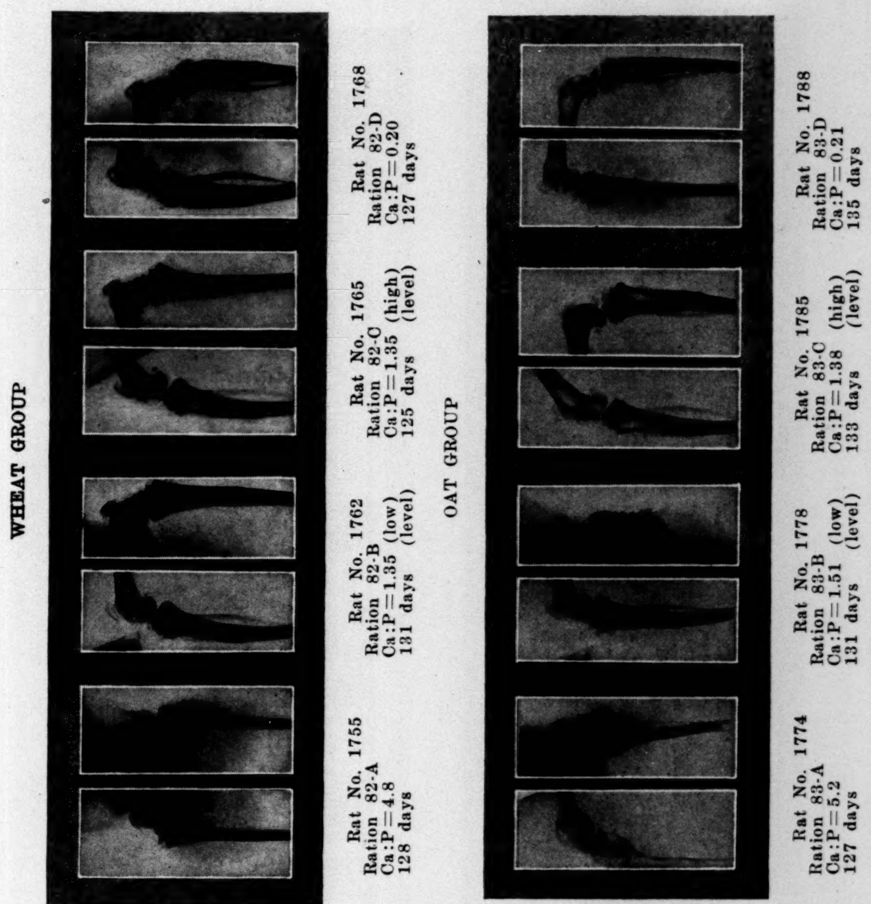

Fig. 2. Illustrating appearance of bone on diets containing 60% of wheat and oat flakes, characterized by different calcium:phosphorus ratios. A calcium:phosphorus ratio approaching unity exerts a pronounced protective effect over a wide range of absolute intake levels of these elements.

In general the information developed by X-ray examinations indicates normal bone formation in the B and C groups of both the wheat and oat series, where the calcium: phosphorus ratios approached unity, regardless of whether the calcium and phosphorus consumptions were on a high or low level. In the A groups where the calcium: phosphorus dietary relationships were markedly dis-

proportionate, there was pronounced development of rickets, more severe in the oat than in the wheat group. In the wheat group also there is evidence of spontaneous healing, whereas spontaneous healing in the corresponding oat group was less readily discernible.

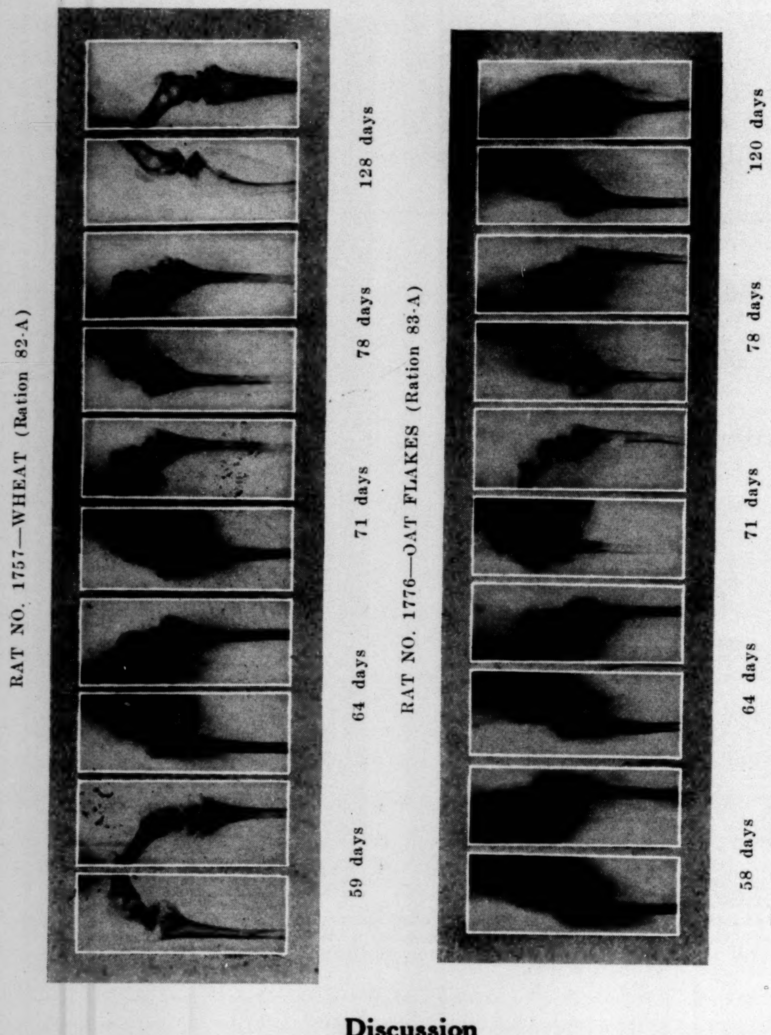

Fig. 3. Illustrating progressive bone changes on rachitogenic diets containing 60% wheat and oats respectively. The wheat series indicates definite tendency toward spontaneous healing. This tendency is less in evidence in the oat series.

Discussion

Briefly, the foregoing data lead to the conclusion that with the rachitogenic rations used in these experiments, wheat contains sufficient vitamin D to permit spontaneous healing if observations are carried over a sufficient period of time. There is less tendency toward spontaneous healing on a comparable oat diet which sug-

gests a lower vitamin D content in the latter grain. In both the wheat and oat series, further illustration is afforded of the regulative effect of appropriate calcium : phosphorus ratios. With such dietary adjustment, rickets does not appear so far as judged by X-ray examinations.

WHEAT GROUP—Ration 82-A

Rat No. 1753 128 days

Rat No. 1754 128 days

Rat No. 1755 128 days

Rat No. 1756 128 days

OAT GROUP—Ration 83-A

Rat No. 1773 127 days

Rat No. 1774 127 days

Rat No. 1775 127 days

Rat No. 1777 120 days

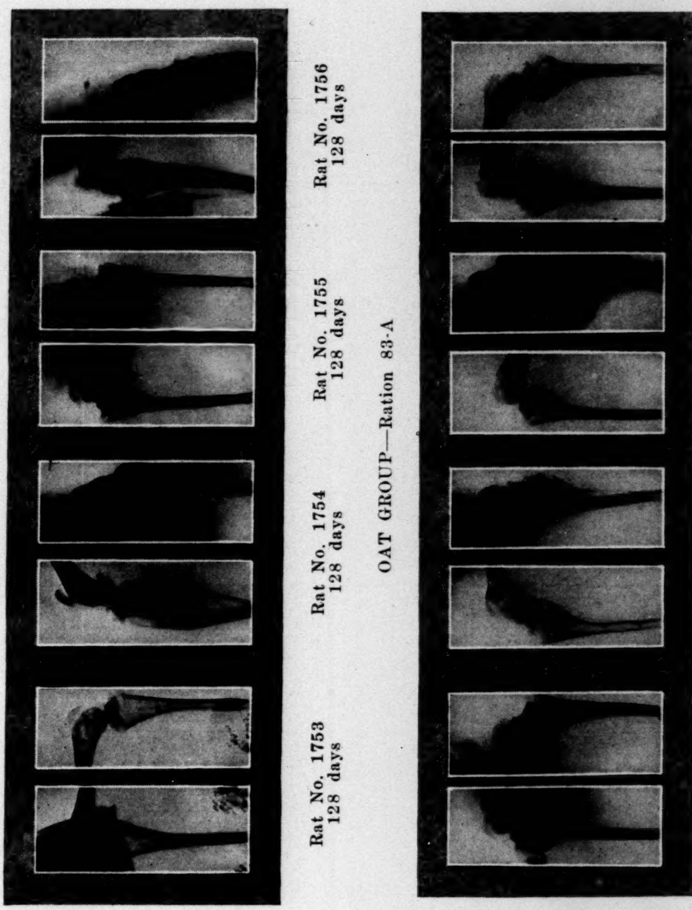

Fig. 4. Illustrating appearance of bone at end of experiment on rachitogenic diets containing 60% wheat and oat flakes respectively.

These data were accumulated during 1925-26. They had not been reported heretofore because of our expectation to further investigate the subject, particularly with reference to exact control of the Ca :P ratios. Inasmuch as the original aim was to compare the effect of extreme disproportions in calcium : phosphorus consumption with ratios approaching unity, it was considered satisfactory to use published values to compute these ratios. The dif-

ferences developed in groups 82-A and 83-A, however, indicated the desirability of specific analytical determination of these ratios and a comparison of the influence of several exactly determined ratios between 5 and 1. Pressure of other work has thus far prevented following through with this plan, and in the meantime the tentative conclusions here reached have received confirmation by other workers. Thus, Steenbock, Black and Thomas (1927) reported that rolled oats is more rachitogenic than wheat and emphasized further the ease with which this defect of the cereals can be remedied by judicious dietary supplementation. In a later publication the same authors (1930) concluded in general that "the cereals

TABLE II

Growth, Food Consumption and X-Ray Details, Wheat Series

	Rat No.	Initial Weight	Weight Gain	Aver. Daily Food Intake	X-Rays Days	X-Rays Description (See also Figs. 2, 3 and 4)
		gms.	gms.	gms.		
Ration 82-A	1753	52	115	7.2	128	All show moderate
Started	1754	58	132	7.1	128	rickets with evidence
9-30-25 and	1755	39	94	5.9	128	of healing.
extending	1756	61	127	7.4	128	
131 days						
	1757	41	120	6.0	59	Severe rickets during
					64	early stages followed
					71	by definite healing
					78	tendencies.
					128	
Ration 82-B	1758	59	171	8.3	132	All almost normal.
Started	1759	69	199	10.2	78	
9-30-25					131	
extending	1760	50	205	10.2	131	
131 days	1761	54	190	9.0	131	
	1762	65	179	10.0	131	
Ration 82-C	1763	59	197	9.5	133	All almost normal.
Started	1764	42	190	8.5	133	
9-30-25	1765	57	192	8.4	78	
extending					125	
138 days	1766	79	184	9.6	133	
	1767	80	196	10.2	133	
Ration 82-D	1768	58	164	8.0	127	All almost normal.
Started	1769	42	155	6.9	127	
9-30-25	1771	45	142	6.7	58	
extending					64	
138 days					71	
					78	
					135	
	1772	51	143	7.8	135	

ranked in antirachitic potency in the descending order of wheat, rolled oats, corn."

Additional confirmation of antirachitic differences between wheat and oats are suggested in the recent paper by Burton (1930), recording observations on children and adults, wherein it is noted

TABLE III

GROWTH, FOOD CONSUMPTION AND X-RAY DETAILS, OAT SERIES

	Rat. No.	Initial Weight	Weight Gain	Aver. Daily Food Intake	X-Rays Days	X-Rays Description (See also Figs. 2, 3 and 4)
		gms.	gms.	gms.		
Ration 83-A	1773	49	73	5.4	127	Very severe rickets
Started	1774	37	75	5.0	127	with relatively little
9-30-25	1775	50	64	5.2	127	evidence of healing.
extending	1777	48	86	5.6	120	
131 days						
	1776	34	51	5.0	58	Very severe rickets
					64	throughout period
					71	with relatively little
					78	healing tendencies.
					120	
Ration 83-B	1778	46	115	7.3	131	All almost normal.
Started	1779	36	160	7.6	131	
9-30-25	1780	32	152	6.7	131	
extending	1781	45	152	7.7	78	
131 days					131	
	1782	50	179	8.7	131	
Ration 83-C	1783	47	194	8.8	133	All almost normal.
Started	1784	50	183	9.1	133	
9-30-25	1785	44	179	8.7	133	
extending	1786	51	193	8.8	133	
131 days	1787	43	183	8.6	78	
					133	
Ration 83-D	1788	45	69	5.4	135	All almost normal.
Started	1789	32	60	5.4	135	
10-1-25	1790	49	88	5.4	58	
extending					64	
130 days					71	
					78	
					135	
	1791	31	115	5.5	135	
	1792	40	93	5.0	135	

that calcium and phosphorus retentions were higher on the wheat than on the oat diet.

Steenbock, Black and Thomas (1930) observed that the oat diet is consumed with more difficulty and in less amount than the wheat diet. Data recorded in Tables II and III show similar differences. This lower level of food intake may to some extent ex-

plain the differences in growth curves. As Steenbock and his associates point out, some deficiency in vitamins B and G may possibly be involved. The relatively poor growth in the oat series would tend to diminish the rachitic effect, whereas on the contrary with the rachitogenic oat diet there was less tendency towards spontaneous healing.

The above described differences between wheat and oats are in keeping with observations of Mellanby. The author, however, is inclined for the present to ascribe such differences to variations in vitamin D content rather than to differences in content of substances exerting a positive anti-calcifying effect as is held by Mellanby.

The practical dietary evaluation of the grains, as of any food product, is based upon their nutritional status with respect to the well-balanced diet. Present knowledge of nutrition discloses numerous important articles of diet which are particularly good with respect to certain nutritional requirements but deficient with respect to others. In fact it is doubtful if any article of diet can by itself be considered nutritionally adequate. With attention to their specific contributions, however, dietetic one-sidedness can be largely avoided. Indeed Cowgill and associates (1927) by appropriate use of supplementary foods, found that when whole grain cereals furnished as much as 84% of the calories of the diet, excellent growth, reproduction, lactation, and general physiological wellbeing could be induced in the rat. Cowgill further stated that "In no cases whatever were any signs of rickets observed in these animals. When it is appreciated that to a certain extent rickets may be regarded as a phenomenon associated with rapid growth, and that these animals showed an unusually rapid growth, the absence of rickets may be taken as even more indicative of the remarkable biologic value of the rations."

Summary

Rats were fed for long periods on diets containing 60% whole wheat, and 60% oats with suitable variations in the calcium: phosphorus ratios in the two series. By means of X-ray examinations progressive bone changes were observed. It is concluded that occurrence or non-occurrence of rickets on both these grain diets can be controlled by proper adjustment of the calcium: phosphorus ratio within wide limits of consumption levels of these elements. Evidence of spontaneous cures on rachitogenic wheat diets is presented. With a comparable oat diet there is less tendency towards

such spontaneous cure. Growth and food consumption were better on the wheat than on the corresponding oat diets. These differences are in harmony with data reported by Mellanby, by Steenbock, Black and Thomas, and by Burton.

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A METHOD FOR TESTING FOR ROPINESS OF BREAD

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Introduction

Rope, an infection of bread by an organism, B. mesentericus, whose spores survive baking temperature, has long been a dread of the baking industry. For the last ten years we have done considerable work for a number of small and some large bakeries. In the course of this work the opportunity has frequently presented itself

to study the conditions under which this so-called bread disease occurs, and to attempt to control it.

The symptoms are too familiar to require detailed description. The odor of an infected loaf, the yellowish discoloration in the advanced stages, the silvery threads forming in the loaf when broken, and the soft, sticky and clammy crumb, form a picture known to every experienced baker. Bread infected by this organism will on hot, damp, summer days develop these symptoms to varying degrees in from 36 to 72 hours. While scrupulous cleanliness, disinfection, and thorough removal of all infected material from the plant is helpful, the only positive and immediate remedy seems to be the use of acids. Vinegar, lactic acid, a mixture of the two, or calcium acid phosphate are the favorite means of creating in the dough a degree of acidity incompatible with the growth of the organism. A dough with a pH between 4.5 and 5.0 appears to be safe.

Owing to the great losses which may be incurred if this condition is not promptly controlled, a great deal of work has been done on studying the source of infection and on testing ingredients as well as the finished product for rope. The best early paper on ropiness in flour and bread is that of E. J. Watkins, read before the Society of Chemical Industry in 1906. A more recent summary of work in this field can be found in a paper by Fisher and Halton (1926). Watkins worked out a practical test for rope in flour which has found very extensive use. We have used this method for years in this laboratory—not entirely with success. There are two reasons for our dissatisfaction with this method. One is that it requires a bacteriologist of considerable skill to avoid unintended infection of the material to be tested. Furthermore, the appearance of the inoculated and incubated "fingers" of bread is not such as to show definitely the presence or absence of rope in any but very pronounced cases.

We have found it more satisfactory to bake up sample loaves with the flour in question, working as cautiously as possible to avoid contamination. The sample loaves were incubated at 30°C. at high humidity. Of the three loaves baked, one was examined at the end of 24 hours, one at the end of 48 hours, and one at the end of 72 hours. The ingredients in the sample loaves were those used by the average baker in making homemade bread. This procedure is similar to that followed by Fisher and Halton.

While the general belief prevails that among the ingredients

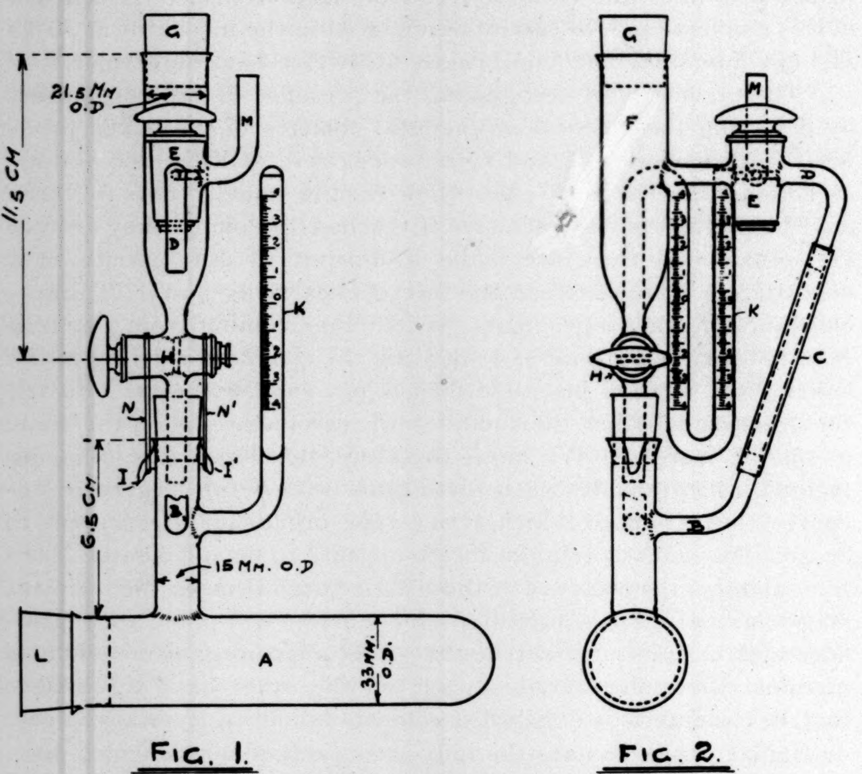
flour is the principal if not the only carrier of this infection, our present knowledge of the field indicates that other ingredients may be involved. We have, therefore, reduced our procedure to a minimum of ingredients when the flour itself had to be tested for rope organisms. In such experiments we make up biscuits instead of loaves of bread. For these biscuits only flour, water, and baking powder are used. The baking powder has previously been tested with flour known to be free from rope to insure its safe use in this connection. The biscuits are placed in the incubator at 30°C. at high humidity, and one broken at twelve-hour intervals.

The procedure of recognizing the presence of rope organisms by detecting the physical or chemical changes in the baked product through odor, feel, and appearance, have at least two serious objections. In the first place they require usually two to three days before a definite conclusion is reached. Secondly, they are not very exact and therefore make it difficult to differentiate in a quantitative sense between the infection-carrying power of different flours or other ingredients. When bread coming from a bakery is tested for the purpose of evaluating the condition existing in the bakery, the method just described is not sufficiently exact to tell the observer whether the condition of infectedness is on the wane or on the increase. We have, therefore, developed the following method which enables us to determine with a fair degree of accuracy the extent to which active rope organisms are present in bread. The method being accurate appears to be sufficiently sensitive to detect the presence of these active organisms in the incipient stages, at a stage when neither odor nor feel will prove their presence with certainty. It is true that other micro-organisms will also manifest themselves in this way; on the other hand we believe that B. mesentericus or allied organisms producing typical ropiness or similar symptoms are the only ones present which under practical conditions will survive baking temperatures.

The Catalase Method for Testing Bread

Catalase is an enzyme present in certain animal tissues and more generally in plant tissues, capable of bringing about the rapid decomposition of hydrogen peroxide to water and molecular oxygen. The presence of this enzyme in bacteria has been studied long ago. The extent of catalase activity in biological material has been measured either by determining the undecomposed hydrogen peroxide, measuring the volume of oxygen produced or deter-

mining the change in pressure as a result of the liberated oxygen C. O. Appleman devised a very good method for catalase measurements in plant tissues. Later on Appleman studied catalases in potato plants by using the simplified form of Bunzell's oxidase apparatus. Fresh bread just out of the oven, infected or otherwise, shows no measurable catalase activity. On incubation, however, as the rope organisms develop, catalase activity becomes pro-



Apparatus for Determining Catalase Activity as a Measure of Ropiness in Bread.

nounced. Our aim in connection with this investigation was to develop a simple apparatus applicable to bread pulp, thereby measuring the extent of catalase activity and thus the extent of ropiness. After some experimentation we decided on the form of apparatus shown in Figure 1 and Figure 2.

Procedure

Twenty-five grams of bread pulp are ground with 50 cc. of tap water and made up to 75 cc. with tap water. By holding the

apparatus at a slant with opening of compartment A tilted upward, the fluid mass is poured with constant stirring into compartment A. The remaining particles of pulp are flushed into the apparatus with an additional 10 cc. of tap water. Stopper L is now inserted tightly. A large glass jar such as a battery jar is filled with water within half an inch from the top. The water should be nearly room temperature (within 1°F.), so that its temperature will not change appreciably during a period of fifteen to twenty minutes. Glass stopcock E is so set as to let air escape or enter through upward bent vent tube M. To hold the funnel more securely in place, stopcock H is attached to glass hooks I and I^1 by means of two rubber bands, N and N^1. The apparatus is now suspended in the water bath by running a glass rod under the upper bend of the manometer tube K and the horizontal tube connecting F with stopcock E. This glass rod will neatly support, therefore, the apparatus at two points and will itself rest on two points on the rim of the glass jar. To establish uniform temperature conditions the apparatus remains in the water bath for a period of ten minutes. (It is best to take the tap water for making up the bread pulp out of the jar containing the water at adjusted temperature.) During the interval of waiting there is introduced into the funnel F 10 cc. of 3% hydrogen peroxide, which has been adjusted to the temperature of the water bath. The hydrogen peroxide fills funnel F almost to the side tube leading to stopcock E. There is also introduced into manometer K mercury to fill both graduated arms up to the points O. Rubber stopper G is inserted tightly into funnel F.

When the ten minutes required for equalization of temperature have elapsed, stopcock E is turned through an angle of 180°, thereby closing all communication of interior of apparatus with the outside but maintaining communication of main body of apparatus with funnel F through rubber tube C. Now stopcock H (immersed under water) is opened, allowing solution of hydrogen peroxide to flow from funnel F into compartment A. This compartment has a capacity of 100 cc. With 95 cc. of liquid introduced there remains a 5 cc. "air bubble" facilitating agitation. The whole apparatus is given a gentle shake or two and the time noted. Readings are made in millimeters on manometer K at intervals of one minute for five minutes.

The Unit of Infection

From the gas space in the apparatus, the change in pressure

and the temperature, it is possible to calculate quite easily the amount of oxygen liberated in five minutes. Tentatively we shall define the unit of infection as that degree of infection which will cause liberation in five minutes of 1 microgram (0.000001 gm.) of oxygen per gram of bread which has been incubated at 50°C. for 24 hours.

Calculations

The volume of the apparatus, when ready for use, measured up to the first 0 point of manometric tube is 135.0 cc. The total volume of liquid used is 95 cc. leaving an air space of 40 cc. In our calculations we consider it safe to disregard the barometric pressure, this introducing a minor and insignificant error. A microgram (0.000001 gm.) of oxygen at temperature t would occupy

$$\frac{32 \times 10^6 \times 273}{22400\ (273 + t)} \text{cc.} \quad \text{or} \quad 0.0007 \frac{273}{273 + t} \text{cc.}$$

In 40 cc. this amount of oxygen would create at 0°C. an additional pressure of 0.0133 mm.

If 25 gm. of bread are used this pressure represents, according to our definition, one twenty-fifth of a unit of infection. One unit of infection would, therefore, theoretically produce at 0°C. in five minutes a pressure twenty-five times as great, or 0.3325 mm.

At 20°C. each unit of infection will produce a pressure of 0.357 mm.

If 25 grams of bread produces too great a reading (over 80 mm.) only one-tenth of the amount or 2.5 gm., or possibly even one-hundredth of the amount, or 0.25 gm., of the material should be used and the results multiplied by ten or one hundred respectively.

Miss Helen Thompson and the senior author are now carrying on experiments determining the optimum temperature for rope development by incubating the loaves at different temperatures. Our results so far indicate that the optimum temperature varies according to the length of incubation. The shorter the incubation the higher the optimum temperature. We believe that for short periods the temperature found will be in the vicinity of 50°C. More accurate data on this point will be submitted in a later paper.

We are also perfecting a procedure which will enable us to determine directly without baking and incubation of bread the extent to which rope-producing organisms are present in flour.

Experimental Data Illustrating Application of Method

The following experiments were performed to establish in a quantitative sense the rope-preventing properties of calcium acid phosphate. Some of the results are here cited to illustrate the manner in which the method can be applied to bakery problems.

Experiment I

Test bakes were made under practically uniform conditions according to our regular laboratory formula. "A" served as control without addition of calcium acid phosphate or other acid material, "B" contained calcium acid phosphate to the extent of 0.5% of the flour, and "D" had calcium acid phosphate to the extent of 1.5% of the flour. All of the flour used was thoroughly infected with a standard sample of rope-infected flour. Of this infected material 0.7 gm. was used per pound of flour. This particular sample of infected flour has been used for such purposes as this for a number of years. It was originally prepared by thoroughly mixing 1 gram of dry and badly ropy crumb with 99 grams of a standard patent flour. Both the infected flour as well as the preventative were thoroughly mixed with the dry batch of flour before addition of water or other ingredients. Sample loaves weighing 1 lb. were allowed to cool for 1 hour, wrapped in paraffine paper and placed in the incubator operating at 30°C. In these particular experiments the three-day incubation at 30°C. was practiced instead of the twenty-four hour incubation at 50°C. as outlined in this paper. This was done to secure data comparable with earlier data in our possession. The resulting data are recorded in Table I.

Experiment II

This experiment differs from Experiment I only in the amounts of preventative used. The results are strictly comparable with those obtained in Experiment I and are recorded in Table II.

TABLE I

Relationship Between Varying Amounts of Calcium Acid Phosphate and the Degree of Infectedness of Incubated Bread as Measured by the Catalase Method

	A	B	C	D
Temperature of Bath	84°F.	84°F.	84°F.	84°F.
Percentage of Preventative	....	0.5%	1.0%	1.5%
Amount of crumb used	0.25 g.	25.00 g.	25.00 g.	25.00 g.
Reading (end of 5 min.)	31	4.0	2.0	0.0
Units of Infection	8,428	11	5	0

TABLE II

	A	B	C	D
Temperature of Bath	84°F.	84°F.	84°F.	84°F.
Percentage of Preventative	....	0.2%	0.4%	0.6%
Amount of crumb used	0.25	0.25	0.25	2.5
Reading (end of 5 min.)	19.0	12.0	4.5	0.0
Units of Infection	5,168	3,264	1,224	0

It is evident from these experiments that the procedure enables us to determine with considerable accuracy the amount of preventative necessary to fully prevent rope development under these conditions. We consider it desirable to disregard all results based on pressure readings of less than 2 mm. at the termination of the experiment. Such slight readings may well be due to increase of vapor pressure, inadequate temperature adjustment, etc., etc. If we disregard such readings (Experiment I, C) we come to the conclusion that an amount of calcium acid phosphate slightly greater than 0.5% based on the flour used, is adequate for rope prevention. Additional experiments would be required to show exactly what this amount is. For our purposes here the above illustrations will suffice.

* * *

This apparatus and accessories can be secured from The Emil Greiner Co., 55 Vandam Street, New York City.

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THE A. O. A. C. GASOMETRIC METHOD FOR THE DETERMINATION OF CARBON DIOXIDE IN BAKING POWDER

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(Read at the Convention, May, 1930)

The A. O. A. C. gasometric method for the determination of carbon dioxide in baking powder was in the form now used, published in 1927.[1] In brief, the method depends on the liberation of the carbon dioxide from a baking powder by the addition of an excess of sulfuric acid and collecting the gas liberated (or its equivalent volume of air). This gas volume is then reduced by calculation to the standard temperature of 0°C. and 760 mm. pressure. Knowing the weight of one cubic centimeter of carbon dioxide under these standard conditions as well as the weight of the sample taken, the percentage of carbon dioxide in the sample can be calculated.

In actual practice however, this method is greatly simplified by weighing exactly 1.7000 grams of the baking powder for the determination. Then when the liberated gas volume has been reduced to standard conditions of temperature and pressure by use of a factor, the per cent of CO_2 in the baking powder is obtained directly by dividing the gas volume obtained by 10.

For simplicity of operation as well as for speed, the official A. O. A. C. gasometric method leaves little to be desired. Especially is this true when comparisons are made with the time-consuming official A. O. A. C. gravimetric (Knorr) method.[2]

The accuracy of the official A. O. A. C. gasometric method has been questioned by Hertwig and Hicks.[3] In their article they say:

"Attention is called to the omission in the Chittick method (A. O. A. C. Book of Methods, 1925, p. 305) for the gasometric determination of carbon dioxide in baking powder, self-rising flour, etc., of a factor to correct the carbon dioxide gas volume reading for the partial displacement due to the vapor tension of the reagent, dilute sulfuric acid, used. This omission under certain circumstances may cause an appreciable error.

"In the execution of this method it is observed that the addition of a measured volume of reagent-acid to the dry decomposition flask causes a greater displacement in the gas-measuring tube

[1] Jour. A. O. A. C. 10: 36 (1927).
[2] Official and tentative methods of analysis of the A. O. A. C., p. 301.

than the volume of added acid. This displacement is greater by about 3 to more than 5 cc., depending on the barometric pressure, the temperature, and the acid concentration, and is attributable to the vapor tension of the reagent-acid."

Before considering in detail this criticism of the official A. O. A. C. gasometric method by Hertwig and Hicks, there is an error in the quoted statement which should be corrected, namely that the method in question is official for baking powder and not for self-rising flour.

Hertwig and Hicks state "the volume reading of the evolved carbon dioxide, in the present Chittick method, consequently requires an empirical correction equal to the difference between the volume of reagent-acid used and the corresponding reading of the displacement effected in the gas-measuring tube after equalization of the internal and external pressures."

According to Hertwig and Hicks this difference amounts to about 3 to more than 5 cc., that is, the volume of gas in the gas-measuring tube is by that amount greater than it should be and it should be subtracted before the gas volume is reduced to standard conditions. In other words if the Hertwig and Hicks suggestion is correct, then the official A. O. A. C. gasometric method gives results which are approximately 0.3% to more than 0.5% too high.

In this article, Hertwig and Hicks give two examples of the analysis of a baking powder for total CO_2 in which the correction as suggested by them is applied, as well as the same analysis in which their correction for vapor tension is not applied. In the latter case the results are 0.6% higher than in the former, which would seem on the face of things to indicate a gross error in the official method.

There is one fundamental principle that should always be observed in the examination of the accuracy of any analytical method, when such a principle can be applied, namely to work with samples, the exact composition of which is known accurately. This cannot be done in all cases but it certainly can be done in the case of baking powders, a compounded product in which the amount and purity of the sodium bicarbonate entering into its composition can be accurately controlled. This is the serious error into which Hertwig and Hicks have fallen: they either did not know or at least failed to report in their paper the total carbon dioxide content of the baking powder which they analyzed. Had they followed this precaution of using for analysis a baking powder of known

[3] Cereal Chem. 5: 482.

strength we believe results would have been obtained indicating the accuracy of the official gasometric method.

There is no question about the accuracy of their statement that when a given volume of dilute sulfuric acid is added to the dry reaction flask, the gas-measuring tube volume will read greater than volume of acid added to the flask, and that this increased volume is due to the vapor tension of the acid. On the other hand, when the actual operation of making a determination for total CO_2 is being carried out, an entirely different set of conditions prevail in the system. The strength of the sulfuric acid does not remain the same, for part of it is neutralized in reacting with the soda of the baking powder, and we have the complex result of the vapor tension of the dilute acid plus the various salts that may be present. This condition undoubtedly does produce a vapor tension but certainly we cannot assume it as being equal to that produced by the acid per se. If there should be an increased volume of gas in the gas-measuring tube due to this vapor tension and actually the analysis of a sample of baking powder gives accurate results without correcting for it, then the conclusion must be drawn that there is some compensating factor. This is actually the case and the compensating factor appears to be the amount of CO_2 that remains dissolved in the liquid in the reaction flask.

Before discussing the study that has been made of the official gasometric method, mention should be made of the fact that all of the equipment used in the work has been standardized for accuracy. This included all volumetric equipment, weights, barometer and thermometers.

The first problem in this study was the preparation of a baking powder of known total CO_2 strength. To this end, the investigation began with the sodium bicarbonate. The bicarbonate was the type used in compounding baking powder on a commercial scale. It may be pointed out in passing that there is no chemical substance produced in a large way, of higher purity than the powdered sodium bicarbonate now obtainable. Triplicate determinations were made of the moisture content by drying to constant weight over sulfuric acid. These determinations gave the moisture content of 0.016%, 0.016% and 0.017%.

This dried bicarbonate was then analyzed for loss of weight by ignition, the change taking place being in accord with the well known equation $2NaHCO_3=Na_2CO_3+H_2O+CO_2$.

The ignition, using 5 gram samples, was carried on in a muffle furnace at a gradually increasing temperature until 560°C. was

reached and maintained for two hours, this temperature being midway between that point at which CO_2 is lost from $NaHCO_3$ and that at which Na_2CO_3 fuses. The losses were 99.86%, 99.86% and 99.83% or an average of 99.85% of the theoretical loss. This data will give an idea of the purity of the sodium bicarbonate used in the subsequent work.

A combination baking powder was prepared, using the dried bicarbonate of the same quality as above mentioned. 57.4 grams of this bicarbonate was made up into a 200 gram sample of the finished baking powder. The acid reacting materials were mono-calcium phosphate and sodium aluminum sulfate. Cornstarch made up the balance. From this amount the finished baking powder would contain theoretically (if the bicarbonate were 100% pure) a total CO_2 content of 15.03%. Applying the factor of purity obtained by ignition, the total CO_2 present was 15.01%.

In order however to get still another check of the total CO_2 present in the baking powder it was submitted to the official *gravimetric* Knorr analysis. The following changes were made in the method which long experience has shown to be necessary in order to get closely agreeing quantitative results. One extra sulfuric acid bulb must be used following the Liebig condenser and two extra potash bulbs for the CO_2 absorption. Bowen potash bulbs were used in place of the Geissler bulbs as mentioned in the official method. Also dilute sulfuric acid was used in place of hydrochloric acid for liberating the CO_2. The sulfuric acid used was made up from one volume of acid to five volumes of water. Objection has been raised to the use of HCl for liberating the CO_2, for the concentration recommended in the Knorr method is such that there is danger of some of the acid being carried over into the train.

Triplicate determinations were made of the baking powder by the Knorr method and the following results obtained for total CO_2: 15.05%, 15.05% and 15.07%, or an average of 15.06%, which figure been raised to the use of HCl for liberating the CO_2, for the con-for analysis by the gasometric method. This result is but 0.05% higher than the result obtained by the ignition method.

The results obtained by four different operators using the gasometric method are given in the following five tables.

The gas volumes were read to 0.10 cc. The correction for volume used throughout all these tests was −0.04 cc.

The average of the forty determinations was 15.079%, a remarkable close check to the theory of 15.06% total carbon dioxide as found by the gravimetric method.

From time to time, determinations were made, as suggested by Hertwig and Hicks, of the increased volume in the gas-measur-

RESULTS OBTAINED BY FIVE DIFFERENT OPERATORS USING THE GASOMETRIC METHOD

TABLE I (By Chittick).

	Gas vol. in cc.	Temp. °C	Bar. reading	% CO_2 found
1	150.46	26.0	752.0	15.19
2	149.96	26.5	751.5	15.08
3	149.26	26.0	751.5	15.05
4	149.46	26.0	751.3	15.07
5	149.96	26.0	751.0	15.12
6	151.66	27.0	744.8	15.07
7	151.96	27.0	744.7	15.10
8	152.56	27.0	744.5	15.15
9	148.16	24.8	753.7	15.09
10	147.96	25.0	753.7	15.05
			Average	15.097

TABLE II (By Dunlap).

	Gas vol. in cc.	Temp. °C	Bar. reading	% CO_2 found
1	149.96	25.0	746.3	15.10
2	151.76	27.0	746.3	15.11
3	149.66	27.0	746.5	14.91
4	150.96	27.0	746.6	15.04
5	150.96	27.0	746.7	15.04
6	149.96	26.5	749.2	15.03
7	150.16	26.0	749.2	15.10
8	149.96	27.0	749.2	14.99
9	149.46	27.0	749.3	14.94
10	149.96	27.0	749.3	14.99
			Average	15.025

TABLE III (By Richards).

	Gas vol. in cc.	Temp. °C	Bar. reading	% CO_2 found
1	148.96	25.0	749.9	15.07
2	148.56	25.5	749.9	14.99
3	148.96	26.0	749.8	14.99
4	148.96	26.0	749.7	14.99
5	149.46	26.0	749.4	15.03
6	149.56	26.0	752.0	15.09
7	149.66	26.0	752.0	15.11
8	149.66	26.0	751.8	15.10
9	149.16	26.0	751.8	15.05
10	149.66	26.0	751.8	15.10
			Average	15.052

ing tube as compared with the volume of dilute sulfuric acid run into the dry reaction flask. This excess volume was found to be from 3 to 5 cc. If the correction as suggested by Hertwig and

Hicks were applied to the determinations made the results would be from 0.3 to 0.5% lower than those obtained and to that extent in error.

In order to determine the amount of dissolved CO_2 in the residual liquor in the reaction flask, the air over the surface of the

TABLE IV (By Preston).

	Gas vol. in cc.	Temp. °C	Bar. reading	% CO_2 found
1.	149.46	23.8	748.0	15.18
2.	149.16	24.1	747.8	15.13
3.	149.46	24.4	747.8	15.13
4.	150.16	24.7	747.2	15.16
5.	149.96	25.0	747.1	15.12
6.	150.06	25.1	746.7	15.11
7.	149.86	25.0	747.2	15.11
8.	150.06	24.7	746.9	15.15
9.	150.26	24.6	746.8	15.17
10.	149.96	24.4	746.7	15.16
			Average	15.142

TABLE V (By Chittick and Richards)

	Gas vol. in cc.	Temp. °C	Bar. reading	% CO_2 found
1.	141.96	15.0	743.0	14.98
2.	141.96	15.0	743.0	14.98
3.	142.66	15.5	743.0	15.02
4.	143.16	15.5	743.0	15.07
5.	142.06	15.0	743.0	14.99
6.	141.96	15.0	743.0	14.98
			Average	15.003

liquid was aspirated for 15 to 30 minutes and then the flask attached to the Knorr method apparatus and the CO_2 dissolved in the liquid determined *gravimetrically*. Converting these weights obtained into volume of CO_2 at some definite temperature and pressure which we might find in the laboratory, the final determinations made gave figures from 2.88 cc. to 3.53 cc. We realize that these figures are perhaps not as accurate as they might be; that during aspiration of the CO_2 from above the liquid some CO_2 may have been lost from the liquid in the flask. At all events, it bears out the point that there is a factor in the CO_2 dissolved in the liquid in the flask which compensates for the vapor tension of the liquid itself when operating at the temperatures employed and stated in Tables I to IV. If this were not so, then there would have to be a correction of some sort applied to the gas volume as

read. That such a correction is unnecessary and that the official gasometric method as now used is one of remarkable accuracy, is fully attested to by the analytical figures obtained. In tables I to IV it will be noted that the temperatures were from 23.8° to 27°C. These temperatures are those not uncommonly met with in a laboratory and yet lower temperatures are also to be considered. In consequence, in Table V are given six determinations made at a considerably lower temperature, namely 15° to 15.5°C. The first three determinations were made by Chittick, the second three by Richards. The average of these six determinations is 14.96% total CO_2 which is 0.10% below the theoretical value. Since the vapor tension becomes lower with lowered temperature and the solubility of the CO_2 in the liquid in the reaction flask would increase, we should expect to find both these factors of vapor tension and solubility working together to decrease the gas volume. The results of analysis obtained at 15°C. indicate this to be the case. While at temperatures between 23° and 27°C. they seem to be completely compensatory. This seems to be shown by the actual figures of the analyses.

Hertwig and Hicks conducted their experiments on baking powder at 22°C. so the results we have obtained cover their working temperature.

It would seem to follow that any CO_2 method which gives results within $\pm$ 0.10% of the actual is a very reliable method.

The authors wish to express their appreciation to Mr. C. J. Preston for his collaboration in this work.

As a matter of record, the humidity of the atmosphere as recorded by the Weather Bureau in Chicago on the days the determinations were made was as follows:

Date	7 A. M.	12 Noon	7 P. M.
February 18, 1930	95	79	75
February 19, 1930	63	54	67
February 20, 1930	77	58	69
March 3, 1930	76	59	65
March 19, 1930	74	40	49
April 15, 1930	67	87	93
April 22, 1930	50	44	41
April 24, 1930	72	54	75

Conclusions

1.—The A. O. A. C. official gasometric method for baking powder gives remarkably concordant results in the hands of different

operators, and results which are very closely in agreement with actual total CO_2 present.

2.—The assertions of Hertwig and Hicks that a correction for vapor tension must be applied is not borne out by our analytical results.

3.—Whatever vapor tension effect is produced by the liquid in the reaction flask is closely compensated for (in those working temperatures generally experienced in the laboratory) by the CO_2 dissolved in the liquid.

VARIATION IN PROPERTIES OF ACETONE EXTRACTS OF COMMON AND DURUM WHEAT FLOURS. A PRELIMINARY REPORT

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Wheat flour contains a relatively small quantity of ether-extractable material, and for this reason probably the fatty constituents of wheat flour have received relatively scant attention from the chemist.

The chemist, however, has given considerable attention to the gluten or nitrogenous compounds of wheat flour and many differences observed in flour quality are quite generally ascribed to differences in gluten quality. Chemical investigations to date, however, have failed to reveal any really significant differences in the chemical constitution of the wheat proteins or in the proportions of the different proteins. Variations in gluten quality, however, do exist and may also be observed in the physical properties of washed glutens from different flours, and are reflected in the quality of baked products from the flour.

It is entirely possible that substances associated with the gluten even in small quantity might appreciably affect the physical properties of the washed gluten. The gluten mass that may be washed from flour is not a purely protein substance, but other substances, particularly fatty, or lipoid materials, are always found to be associated with the proteins.

Dill (1925) found an appreciable quantity of ether-alcohol sol-

uble material associated with wheat protein in dried washed glutens.

Sullivan and Near (1927) determined the lipoid content of several glutens from different types of wheat, finding a variation from 2.30 to 3.84%. The glutens of best quality contained the least lipoid material.

Sullivan and Near in a later article (1928), show the distribution of lipoids and lipoid phosphorus in different mill products. The patent flour contained the smallest percentage of total lipoids, but the phosphorus content of the lipoid material was highest in the patent flour.

Working (1924) showed that the addition of wheat phosphatid and lecithin to bread dough in increments of 0.5 to 1.0% injured the baking quality.

Chemical literature, however, contains very few references to the nature of the fatty material of the wheat kernel. These references also generally refer to fatty material found in the wheat embryo. Ball (1926) however, examined a wheat fat prepared by extracting a patent flour with ether. The wheat fat prepared by Ball from patent flour showed the following constants:

Sp. gravity	0.96729
Saponification No.	160.86
Iodine No.	105.43
Unsaponifiable Material	2.51%

The fatty material extracted by usual fat solvents always contains some unsaponifiable material. Anderson and Nabenhauer (1924) found two phytosterols in wheat endosperm—sitosterol ($C_{27}H_{45}OH$) and dihydrositosterol ($C_{27}H_{47}OH$). The sitosterol of Anderson and Nabenhauer was similar to the material which they obtained from corn and melted at 137.0 to 137.5°.

Gortner (1928) had noted a crystalline material which separated from ether extract of flour. This material, however, was not readily soluble in ether.

Ball (1926) noted a substance which separated from ether extract of flour on standing. This crystalline material had a melting point of 93.0 to 93.5°C. Treatment with boiling alcoholic sodium hydroxide for thirty minutes did not change the melting point, but after five hours treatment with alcoholic sodium hydroxide the substance gave a melting point of 137.5°C. Ball concludes that the material which separated from the ether extract is an ester of sitosterol.

Experimental

As stated previously, very little data is available regarding the nature of the non-phosphatid fatty substances in wheat flour. Ether dissolves a considerable amount of phosphatids and it was desired to secure a wheat fat preparation practically free from lipins or phosphatids. According to MacLean (1918), phosphatids are soluble in different degrees in all of the common fat solvents with the exception of acetone. The phosphatids are practically insoluble in acetone. In preparing the wheat fat for the experiment, therefore, a direct extraction of the flour was made with acetone at room temperature.

It was thought desirable to compare two flours known to have quite different properties. Accordingly about 15 pounds each of the Marquis and Mindum varieties were secured through the Department of Agronomy from the Langdon Sub-Station. The Marquis variety is a common spring wheat, the flour of which is noted for excellent bread-making properties. The Mindum variety is a high grade amber durum which is very desirable for preparation of macaroni products, but is decidedly inferior in bread making quality. The two lots of wheat were milled on the small experimental mill and a 75% patent flour prepared from each. Table I shows the protein and ash content of the two flours and also the per cent of material extracted by ether and cold acetone.

Preparation of Acetone Extracts

A quantity of each of the flours was placed in a bottle of approximately 3 liters capacity and sufficient c.p. acetone added so that the flour was covered about two inches. The flour-acetone mixture was thoroughly agitated and allowed to stand for a week. The solvent was then removed by filtering through a large Büchner funnel into a suction flask. One thickness of fine white cotton cloth and a filter paper were used in the Büchner funnel. The flour on the filter was washed twice with acetone, but while the greater part of the acetone soluble material was thus removed no attempt was made to completely extract the flour.

The solvent was removed and recovered by distillation under reduced pressure. During the distillation a considerable amount of flaky crystalline material separated out from the Marquis wheat extract, but this was not noted in the durum extract. After reducing to small volume, the final traces of acetone were removed by exposing in a beaker in a warm dark place. The oil or fatty

substance from Marquis wheat contained considerable crystalline material, while the durum extract when freshly prepared was clear and also less viscous than the Marquis extract.

Properties of Flour After Extraction With Acetone

Gluten could still be washed from the acetone extracted flour but the gluten differed from normal gluten in being rather stringy and lacking in toughness.

One sample of acetone extracted flour was baked. The loaf from this sample was very inferior in size and texture to the loaf baked from the original flour.

TABLE 1

PROTEIN, ASH, AND ETHER AND ACETONE SOLUBLE MATERIAL OF PATENT FLOURS (Dry Basis)

	Protein in Flour %	Ash in Flour %	Ether Soluble Material %	Acetone Soluble Material %
Marquis	14.0	0.42	1.28	1.25
Mindum	13.1	0.63	1.29	1.26

TABLE 2

NITROGEN AND PHOSPHORUS CONTENT OF ACETONE EXTRACTS OF PATENT FLOUR

	Nitrogen %	Phosphorus %
Marquis	0.113	.090
Mindum	0.100	.090

TABLE 3

COMPARISON OF ACETONE EXTRACTS OF PATENT FLOUR

	Marquis	Mindum
Unsaponifiable Matter, per ct.	4.37	6.01
Free Acid Number	20.11	17.78
Saponification Number	133.5	159.1
Ester Number	113.4	141.3
Iodine Number	92.2	109.3

Properties of Acetone Extracts

Both preparations showed a tendency to harden and become insoluble when exposed to the air in a thin layer. The property will be discussed later in connection with the iodine number.

The nitrogen and phosphorus content of the two acetone extracts was determined and these data are shown in Table II. The nitrogen content may be due to some contamination of protein material, but the low phosphorus content indicates a low phosphatid content. Near and Sullivan (1928) found ether extracts of patent flour to contain more than 0.5% phosphorus. By using acetone as a solvent, therefore, a wheat fat preparation of relatively low phosphorus content may be prepared.

From Table I, it will be noted that acetone dissolves almost as much material as ether, and warm acetone dissolves more. Since the phosphorus content indicates that relatively little phosphorus-bearing fats are dissolved, it is evident that acetone dissolves other substances not readily soluble in ether. The extracts when freshly prepared, however, were completely soluble in both acetone and ether. The acetone extracts do, however, contain a relatively large amount of unsaponifiable matter.

Examination of Extracts

Table III compares the results obtained upon examining the two extracts.

The Mindum extract contained a higher percentage of unsaponifiable matter than the Marquis extract. The Marquis has a higher free-acid number but the principal difference is found in the saponification number and the iodine number. The saponification number for the Mindum is significantly higher and would indicate a lower average molecular weight of fatty acids than found in the Marquis. The ester number is also higher for the Mindum extract.

The iodine number of the durum extract is also significantly higher. This would indicate a higher degree of unsaturated fatty acid. The unsaturated compounds, however, may be partly of the oleic acid type. The durum extract has the highest iodine number, but the Marquis extract shows greater drying properties when exposed in the laboratory.

Unsaponifiable Material

Both extracts gave a positive Liebermann-Burchard reaction for phytosterol. In the case of the durum the color developed immediately, but in the case of Marquis extract the color developed quite slowly. This was surprising since the Marquis extract contained considerable crystalline material.

In order to check up this matter, 500 grams each of (1) commercial bleached patent from hard spring wheat, (2) a Kubanka durum patent and (3) a Quality (common white wheat) patent were placed in percolators and extracted with acetone. The first drippings from the percolators were in all cases colored and after about 400 cc. were collected the flasks under the percolators were removed and replaced with other flasks. The second and third 400 cc. were colorless in all cases, but after standing overnight a crys-

talline material separated out of the first extraction of (1) and (3) but none separated from the durum. It was found upon cooling the second and third extracts in a salt-ice bath to 0°C. that a considerable quantity of white flaky crystalline material separated from the common wheat extracts, but none from the durum. The crystalline material from the common wheat extracts was filtered off, redissolved on filter paper with ether, and the ether removed by evaporation. This material gave a positive Liebermann-Burchard test, but the color formed very slowly.

This material is evidently similar to the substance described by Ball (1926). When recrystallized three times from alcohol, crystals from hard red spring and white wheat patents melted at about 93°C. After eight hours' treatment with boiling alcoholic potassium hydroxide, a substance which melted at 137°C. was obtained. Ball concluded that this compound was an ester of sitosterol. The Liebermann-Burchard test indicates that the durum extract contains a phytosterol. The durum extracts, however, do not contain this sterol found in the common wheat extracts which gives a weak Liebermann-Burchard reaction and is precipitated from acetone solution on standing or cooling.

Discussion

It is realized that the data presented in this paper is not sufficiently extensive to permit definite conclusions. The work must be repeated on additional samples to determine if differences noted are significant and consistent.

The variations noted, however, are of interest and suggest that the role of non-phosphatid fatty substances may be quite important in wheat flour. Presuming that variation in quality of gluten is due to variation in nature and amount of fatty materials associated with the proteins, the following observed flour phenomena may be explained by such a theory, namely:

(1) The water absorbing capacity increases and the baking quality of flour improves upon aging or storage.

(2) Certain bleaching agents in addition to effect on color, also affect baking quality.

(3) Oxidizing agents such as potassium bromate, when added to the dough have a pronounced effect on baking quality.

(4) Kent-Jones (1927) has recently observed that the baking quality of flour may be improved by heat treatment.

All of these changes might be due to oxidation of the unsaturated fatty materials associated with the gluten. Further in-

formation on the nature of the unsaturated materials present is desirable.

The presence or absence of certain forms of sterols may have an important effect on gluten quality.

A sterol is present in common wheat flour which is evidently not present in durum. This sterol, which is probably an ester of sitosterol, is relatively insoluble in acetone at temperatures under 10°C.

Summary

1. The acetone extracts of wheat flour are comparatively low in phosphorus content.

2. The durum extract has a higher saponification number and ester number than the common wheat extract.

3. Both extracts show to some extent the properties of drying oils. The durum wheat extract has the highest iodine number.

4. A sterol is present in common wheat which is relatively insoluble in acetone at low temperatures.

Acknowledgment

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PROTEOLYSIS IN BREAD DOUGHS[1]

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(Read at the Convention, May, 1929)

When bread doughs are fermented with yeast certain physical and colloidal changes are obviously taking place. These changes are frequently spoken of as "ripening" of the gluten and are generally attributed to a change in the gluten proteins. One not infrequently hears the expression "the gluten is broken down." Immediately the question arises as to just what is meant by this "breaking-down" process. Colloidal and physical changes can be readily detected simply by the handling of the dough as fermentation progresses, and in this way an experienced baker can judge more or less accurately the "progress" of the dough; conversely, the chemical changes, if any, in the structure or degree of aggregation of the gluten proteins that accompany this so-called "ripening" process are not so easily followed.

For many years considerable attention has been devoted to the proteoclastic activity of flour in view of the possibility that it is a very important factor in determining the value of the flour for bread-making purposes. During the bread-making process yeast is added to the dough. Just what effect this may have on increasing the proteolytic activity is not definitely known. Thus we have two possible sources of proteolytic enzymes in ordinary bread doughs, but the actual extent to which proteolysis takes place during fermentation is still imperfectly understood.

With these considerations in mind, the purpose of this study has been to find some means of demonstrating the chemical changes in the gluten proteins that accompany these physical and colloidal alterations, and subsequently determining to what extent these phenomena take place.

The Material and Its Preparation

The observations in this study have been confined to standard patent and clear flour doughs fermented for varying lengths of time up to 5 hours. These flours on analysis were found to have the following composition:

	Crude Protein (N x 5.7) %	Ash %
Standard patent flour	11.41	0.48
Clear flour	13.93	1.60

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[2] Fleischmann Research Fellow.

The doughs were mixed with the following formula:

Flour	300 grams
Yeast	9 grams
Sugar	7.5 grams
Distilled water	sufficient

The quantity of flour indicated, namely 300 gm., was actually adjusted on the basis of 15% moisture content. It will further be noted that common salt or sodium chloride was not included in the dough formula for reasons that will later become apparent.

Freezing Methods.—In order to study the changes that occur during the fermentation period, it became necessary to find some means of arresting the action of the enzymes. To accomplish this, aliquots of the dough were taken at varying intervals and frozen solid in a refrigerator maintained at $-17 \pm$ °C. At this low temperature the doughs, being spread in a thin sheet, are rapidly cooled, thus inhibiting enzyme action very shortly after the doughs are placed in the refrigerator, even though $25\pm$ minutes are required for freezing the doughs solid.

The exact procedure used in preparing these doughs was as follows:

The doughs were mixed in the Fleischmann mixing machine for 3 minutes. The dough was immediately weighed and one-third by weight was removed and frozen. This was accomplished by spreading the dough in a thin sheet on a plate glass slab which was at once placed in the refrigerator maintained at $-17\pm$°C. Under these conditions the dough was frozen solid in $25\pm$ minutes.

The remaining two-thirds was placed in a closed vessel in an air thermostat maintained at 30°C., and allowed to ferment for 2.5 hours. At this time one-half this portion of the dough was removed and frozen in the manner indicated above. The remaining portion of the dough was fermented an additional 2.5 hours, or a total of 5 hours, and was then frozen in like manner.

It was necessary to conduct several experiments directed toward the development of a convenient and satisfactory method for effecting the dispersion of the dough in a solvent for peptization or other manipulations. If the slab had been greased the sheet of dough could be completely removed by slipping the edge of a chisel between it and the glass. The frozen material was then cracked into fragments and placed in the hopper of a meat grinder. When the handle was turned in the reverse direction to that which is usual to such a device the frozen dough would be crushed against the rear wall. After 15-20 revolutions of the grinder the device was

removed from the table and the fine powder or meal transferred to a beaker by inverting the grinder. The material thus produced was sufficiently fine and lent itself readily to dispersion in a solution or solvent to be used for study.

Methods and Results

An actual determination of the relative protease activity that takes place in an ordinary bread dough has proved difficult. This is due in no small measure to the complex nature of the flour proteins, and more particularly to the fact that seemingly small modifications of the structure of the gluten proteins may be accompanied by substantial alteration of their colloidal behavior. In studying proteolytic action as a whole one is not in want for methods; in general, however, each method has been developed for a particular kind of investigation on a specific material, and in many cases they are not adaptable or useful in studies on material of a different nature. Cairns and Bailey (1927) and Olsen and Bailey (1925) have made reference to the inadequacies of the available protease methods.

Recently Gortner, Hoffman and Sinclair (1929) have called attention to the substantial variations in the proportion of total protein dispersed or peptized in various saline solutions. It appeared possible that the use of certain of these saline solutions might result in the disclosure of changes in the structure or degree of aggregation of the flour proteins which are effected by the proteases active in an ordinary bread dough. To test this assumption the following experiments were undertaken.

A portion of this frozen dough-meal or powder was weighed off which was equivalent to 6.0 grams of flour, containing the equivalent of 15% moisture. In this way the same proportion of flour appeared in each of the extracts or preparations. This material was at once suspended in 50 cc. of a 0.5 M saline solution.

Three saline solutions were employed in this work, namely 0.5 M K_2SO_4, 0.5 M $MgSO_4$ and 0.5 M $MgCl_2$. In order to have equal ionic concentrations of all salts, 0.5 M solutions were used throughout instead of solutions of the per cent by weight most commonly used. The saline solutions were prepared in a more concentrated form than that in which they were actually used. Thus the $MgCl_2$ and $MgSO_4$ solutions were equivalent to 1.0 M while the K_2SO_4 stock solution was 0.6 M, since the latter salt is not sufficiently soluble in water to yield a solution as concentrated as

1.0 M. In all three cases a sufficient quantity of the stock solution was used to contribute the equivalent of the salt in 50 cc. of 0.5 M. To this was added a quantity of water which, added to the water in the dough, would bring the volume of the stock solution up to 50 cc. and thus render the solution 0.5 M.

The frozen dough-meal was suspended in the saline solution in a centrifuge tube. The tube was then placed in the centrifuge and whirled rapidly for 10 minutes to precipitate all suspended particles. The clear liquid was decanted into a Kjeldahl flask. To the residue in the centrifuge tube was added another 50 cc. portion of the same saline solution (0.5 M concentration), the residue suspended by vigorous stirring with a glass rod and shaken for another 30 minute period. The suspension was then centrifuged and decanted as before, the decantate being added to that of the first extraction. This process of re-extraction was repeated a third time. The combined extracts were then subjected to the usual Kjeldahl-Gunning method for the determination of protein nitrogen. Since the total protein content of the original flour was known it was possible to compute the quantity of protein dispersed by these various saline solutions in terms of the percentage of total protein present, and the data reported are all recorded on this basis.

As has been indicated by Gortner, Hoffman and Sinclair (1929), the proportion of the total protein dispersed by these three salts in the concentrations used differed appreciably. They found the proportion of protein peptized from 12 samples of flour to average the least in the instance of K_2SO_4 solutions and the greatest in the case of the $MgCl_2$ solutions, as far as the three salts employed are concerned. This same difference in the peptizing effect of the three saline solutions was observed in the instance of the two flours which were used, although there was less difference between the K_2SO_4 and the $MgSO_4$ solutions than would be anticipated from the averages obtained by Gortner et al with the 12 flours that they examined. In view of the large variations of different flours upon such treatment it is not difficult to see the possibility of such differences when using only two flours as criteria.

Saline Peptization of Flour as Compared to the Freshly Mixed Dough.—In the early stages of this work it seemed logical to make a comparison between the peptizing effect when the saline solutions were applied to the freshly mixed unfermented dough and to the flour itself. The resulting data are given in Table I, and are recorded graphically in Figure 1.

TABLE I

PERCENTAGES OF TOTAL PROTEIN EXTRACTED FROM FLOUR SAMPLES WITH 0.5 M. SALT SOLUTIONS AS COMPARED TO THAT EXTRACTED FROM THE FRESHLY MIXED DOUGHS OF THE SAME FLOUR

Salt Solution	Clear Flour	Clear Dough	Standard Patent Flour	Standard Patent Dough
$MgCl_2$	51.40	41.27	32.16	35.85
$MgSO_4$	37.59	25.84	18.18	16.24
K_2SO_4	35.53	24.97	15.58	14.54

TABLE II

PERCENTAGES OF TOTAL PROTEIN EXTRACTED FROM FROZEN DOUGHS WITH THREE 0.5 M. SALINE SOLUTIONS

Hours Fermentation	$MgCl_2$ 0.5 M	$MgSO_4$ 0.5 M	K_2SO_4 0.5 M
Standard Patent Flour Dough			
0	35.85	16.24	14.54
2½	36.50	16.16	16.08
5	36.06	18.22	16.16
Clear Flour Dough			
0	41.27	25.84	24.97
2½	41.58	24.56	27.13
5	41.09	28.03	27.84

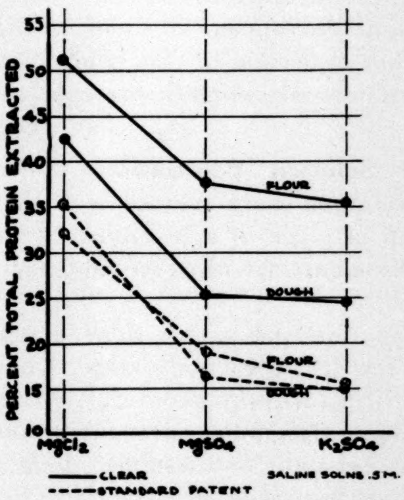


Figure 1. Percentages of total protein extracted from flour samples with 0.5 M. salt solutions as compared to that extracted from the freshly mixed doughs of the same flour.

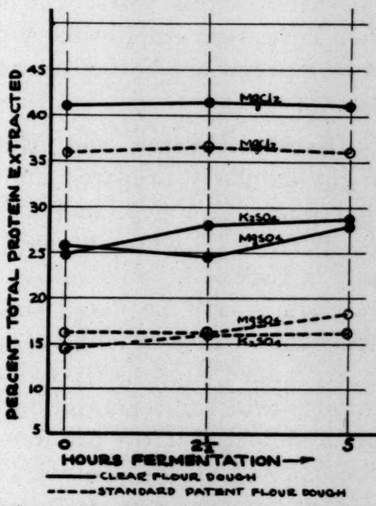


Figure 2. Percentages of total protein extracted from frozen doughs with three 0.5 M saline solutions.

It will be observed that the differences between the standard patent dough and the standard patent flour were relatively small in view of the large experimental error of the method. In case of

the clear flour a much larger percentage of the protein was peptized by all the solvents when they were applied to the flour than when applied to the freshly mixed dough. The reason for this difference is the case of the low grade flour is not apparent from any observations in these experiments.

Saline Peptization of Doughs Fermented Varying Lengths of Time.—Having standardized the procedure in the instance of the peptization of doughs the method was then applied to doughs fermented 0, 2.5, and 5 hours respectively. The resulting values, in the instance of doughs made from the two grades of flour, are recorded in Table II, and are shown graphically in Figure 2.

It will be observed that no tangible evidence of any substantial modification of the protein molecule was revealed by the degree of peptization as evident from these data. The small differences noted are probably within the range of experimental error of the method, which is rather large in dealing with such material as dough. This was also true in the instance of clear flour dough, where it might be assumed that protease activity would be at a higher level than in the standard patent doughs. It is conceivable that certain compensating variables are operating in the instance of the saline solutions used in this work which might not appear if some of the other salts were employed. A further extension of this study may reveal a procedure better suited to a demonstration of protease activity.

Distilled Water and Buffer Solution Peptization.—When doughs similarly prepared and fermented were treated with distilled water as a peptizing medium in place of salt solutions the data in Table III were obtained. These data are represented graphically in Figure 3.

Here it will be noted that the standard patent flour dough yielded about 28% of its protein to the distilled water extract when freshly mixed, and nearly twice this quantity or about 52% of its protein to the same extraction medium after 5 hours fermentation. It is assumed that the difference in behavior of these two doughs is occasioned by the operation of two variables at least, these being (1) the change in H-ion concentration, and (2) the precipitating effects of certain salts or electrolytes in the dough solution. Bailey and Sherwood (1923), and Johnson (1925) have shown that high grade or patent flour doughs change in H-ion concentration much more rapidly than the lower grade or clear flour doughs, due to the higher concentration of buffering substances in the latter.

From these data, then, one has reason to believe that more of the proteins are peptized at the higher H-ion concentration encountered in the fermented patent flour dough than in the freshly mixed dough.

Some indication of this effect of H-ion concentration was afforded by the results of a parallel experiment in which a phosphate buffer solution (pH = 6.8) was used as the extraction medium in place of distilled water. This solution was prepared by mixing a 4% monobasic sodium phosphate solution with a 4% dibasic sodium phosphate solution until pH = 6.8 was reached. Of course it is recognized that such an experiment does not establish the role of the H-ions in a definite and clean-cut manner, since the phosphate and other ions of the buffer solution may effect a precipitation of the dispersed proteins. Likewise, certain ions in the clear flour (aside from H-ions) may depress the peptization of the clear flour dough proteins when treated with distilled water. Thus the buffer solution used in the peptization of the standard patent flour dough (Table III) tended to simulate the conditions prevailing when clear flour dough is extracted with distilled water in the direction of coincidently buffering the solution and introducing certain ions which may serve to precipitate proteins that would otherwise be peptized as in the standard patent flour dough solution.

Acetone Dehydration of Doughs.—While the extraction of the proteins of frozen dough proved feasible, this procedure was not convenient and entirely desirable. Such material can only be preserved in a frozen state and all of the observations or tests must be applied immediately after the material is again thawed. A procedure which would enable the analyst to preserve the important fractions of the dough for indefinite periods would have obvious advantages.

In other phases of biological work it has been found that moist tissues can frequently be dehydrated with acetone and subsequently brought into a fine state of subdivision for extraction or other manipulation. It appeared possible that a like procedure might be developed for use in the study of fermented dough. In developing this method it appeared desirable to arrest fermentation by freezing the dough, and subsequently grinding this dough into the acetone, which reagent was then afforded an opportunity to remove the major portion of the water in the dough. A preliminary experiment was conducted to determine the quantity of residue that would remain after treatment with acetone.

TABLE III

PERCENTAGE OF TOTAL PROTEIN EXTRACTED FROM FROZEN DOUGHS BY DISTILLED WATER AND A SODIUM PHOSPHATE BUFFER SOLUTION (pH=6.8)

	Hours Fermentation	Distilled Water	Buffered Solution
Standard Patent Dough—			
	0	27.72	20.20
	2½	32.55	20.24
	5	51.92	18.09
Clear Flour Dough—			
	0	24.78	
	2½	23.90	
	5	24.33	

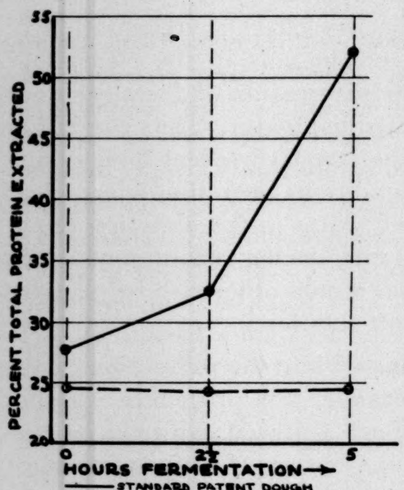


Figure 3. Percentages of total protein extracted from frozen doughs by distilled water.

A quantity of the dough equivalent to 6 gm. of the original flour was weighed from the frozen and powdered material. This frozen dough-meal was placed in a 100 cc. beaker and 75 cc. of 99% acetone added. This mixture was allowed to stand in the refrigerator at a temperature of –17±°C. for 24 hours, being stirred occasionally during the early part of the period. The acetone was then carefully decanted and the dough residue collected on a watch glass and dried in a current of warm air for 30 to 45 minutes. The weights of the residue thus obtained from both the standard patent flour dough and the clear flour dough are recorded in Table IV.

TABLE IV

RESIDUAL WEIGHTS OF DOUGHS EQUIVALENT TO 6 GM. OF ORIGINAL FLOUR AFTER DEHYDRATION WITH ACETONE

Hours Fermentation	Clear Flour Dough	Standard Patent Dough
0	6.00	6.03
2½	6.01	6.00
5	6.00	5.99

It will be noticed that the largest variation from 6.00 gm. obtained from both the standard patent and clear flour dough was not more than 0.03 gm. It was accordingly concluded that a 6 gm.

charge of dehydrated dough would be approximately equivalent to 6 gm. of the flour computed on a 15% moisture basis.

The acetone which had been in contact with 172 gm. of dough for 24 hours was placed in a Kjeldahl flask and the nitrogen determined in the usual manner. Determinations on the acetone from two different dough samples yielded titrations of 0.5 and 0.6 cc. of N/14 acid, including the reagent blank of 0.4 cc. Considering the large quantity of dough subjected to each manipulation, it was concluded that the amount of nitrogen removed by the acetone was negligible.

On the basis of these preliminary experiments, the following procedure was adopted:

> Aliquots of dough which had been fermented for varying periods of time were frozen at –17±°C., and ground in the frozen state with a meat grinder as before. This ground, frozen dough-meal was placed in a beaker and 800 cc. of 99% acetone added. This mixture was allowed to stand in a refrigerator at –17±°C. for 24 hours with occasional stirring. The acetone was then decanted and the residue collected and dried in suitable shallow containers. The dry material was then ground to the fineness of flour by passing it several times through a burr mill or until it was possible to pass it through a 10XX flour bolting cloth.

Material thus prepared could be subjected to treatment in essentially the same manner as the original flour.

Peptization Experiments on Acetone Dehydrated Doughs.—A series of doughs was then dehydrated and subjected to study in the same manner as in the instance of the "frozen dough" experiments, with the data recorded in Tables V and VI, and graphically shown in Figures 4 and 5.

Figure 4 should thus be compared with Figure 1, and it becomes apparent that the several saline solutions extract almost identically the same proportion of the total protein in the acetone-treated dough as was extracted from the frozen doughs. It will be noted that by this treatment the same differences occur between the clear flour and clear flour dough as in the case of the frozen doughs.

In the studies of the effect of fermentation (Table VI and Figure 5) some differences appear between the acetone treated doughs and the frozen doughs (Compare Figure 2).

TABLE V

Percentages of Total Protein Extracted from Flour Samples with 0.5 M Salt Solutions as Compared to That Extracted from the Freshly-Mixed Acetone-Dehydrated Doughs

Salt Solution	Clear Flour	Clear Dough	Standard Patent Flour	Standard Patent Dough
$MgCl_2$	51.40	42.49	32.16	35.36
$MgSO_4$	37.59	25.26	18.18	16.17
K_2SO_4	35.53	24.55	15.58	14.98

TABLE VI

Percentages of Total Protein Extracted from Acetone-Dehydrated Doughs Which Were Fermented Varying Lengths of Time

	Hours fermentation	$MgCl_2$	$MgSO_4$	K_2SO_4
Standard Patent Flour Dough—				
	0	35.36	16.17	14.98
	2½	31.54	13.53	11.73
	5	32.99	16.16	14.50
Clear Flour Dough—				
	0	42.49	25.26	24.55
	2½	36.85	23.18	20.63
	5	39.76	25.55	24.15

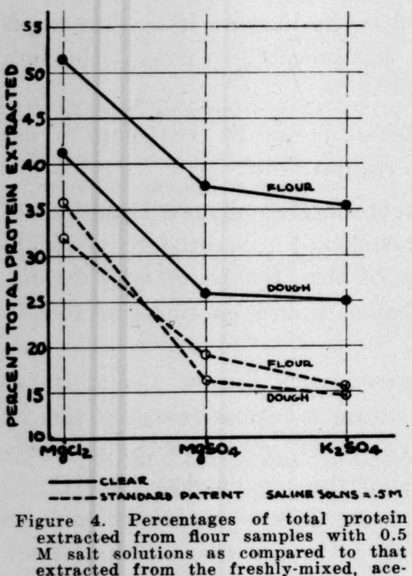


Figure 4. Percentages of total protein extracted from flour samples with 0.5 M salt solutions as compared to that extracted from the freshly-mixed, acetone-dehydrated doughs.

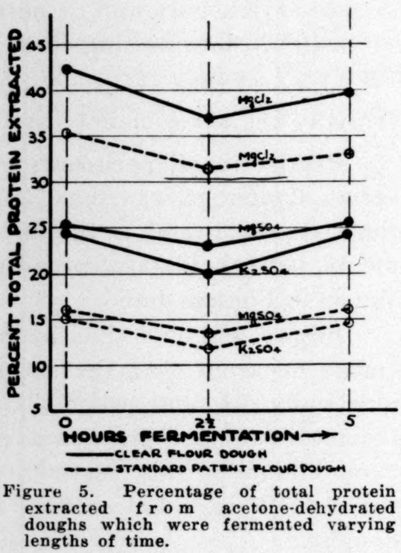


Figure 5. Percentage of total protein extracted from acetone-dehydrated doughs which were fermented varying lengths of time.

The percentage of total protein extracted from dough fermented 5 hours was again about equivalent to that extracted from the freshly-mixed dough as in the instance of the frozen doughs.

For some reason which is not apparent the percentage of total protein peptized in doughs fermented 2½ hours is somewhat less than in the instance of the freshly-mixed doughs. If this had appeared in the case of only one or two of the doughs of the series of six it might have been attributed to experimental error, but since it appears so consistently in all six of the doughs it appears that there must be some cause operating that is not disclosed in these observations.

Disregarding this seeming depression of peptizable proteins at the 2½ hour fermentation interval, the data seem to indicate that again there is no evidence of a substantial modification of the proportion of peptizable proteins in a five-hour fermentation period, in the instance of these acetone-dehydrated doughs.

It appears that this method of preparing fermented doughs for biochemical study may prove very convenient and valuable in various phases of such research. The preparations when made will no doubt be available for use over extended periods of time, possibly indefinitely. The dehydrated dough thus obtained is easily disintegrated to a fine state of subdivision with ordinary grinding appliances, which greatly facilitate the ease and exactness of subsequent extraction and other manipulation.

It is interesting to note in this connection that the yeast cells evidently were not killed by this treatment, for under certain conditions they would induce vigorous fermentation. A series of tubes containing the centrifuged residue from saline extractions was accidently left over night without cleaning. The next morning the tubes that had been extracted for a total of 1½ hours with 0.5 M $MgSO_4$, which one would ordinarily assume to be toxic to the yeast cells, were found to contain the top half of the former residue halfway up the tube. In the case of doughs extracted with $MgCl_2$ and K_2SO_4 no fermentation was observed. This indicates it is the combination of the Mg^{++} and $SO_4^{=}$ ions that stimulated the yeast action, since no fermentation occurred in doughs which were extracted with distilled water. This seems to eliminate the idea that the other salts simply hindered the growth of the yeast. If these tubes were allowed to remain another day all the residue that had not been treated with $MgSO_4$ developed an abundance of common moulds such as Rhizopus and Penicillium. The $MgSO_4$ dough residues, however, would not develop these moulds as the yeast seemed to get ahead of them. These experiments were repeated several

times with identical results, even to the height to which the fermenting dough rose in the tube overnight.

A Study of Doughs with Added Protease.—Ford and Guthrie (1908) demonstrated the deleterious effects of even small quantities of proteolytic enzymes on gluten. It appeared possible that the introduction of such enzymes into dough might raise the proteolytic activity to such a level that it could be easily measured by peptization with these saline solutions. If differences then appeared at various fermentation intervals, one would be convinced that this method is capable of detecting such changes in the protein molecules and also that no similar changes are taking place in the ordinary bread dough to which no protease is added. If, however, no substantial changes were detectable it might mean that this method is not capable of measuring such changes in the protein structure as take place in these doughs, or it might mean that the effect of the added proteases on the chemical structure of the gluten proteins is negligible, although the colloidal and physical properties are substantially altered.

Accordingly three doughs were mixed from the same standard patent flour that was used in previous experiments. Aside from the addition of the enzymes the doughs were prepared in exactly the same manner as before. One 300 gm. control dough was mixed and aliquoted into three equal portions. Two of these portions were baked according to the Standard Experimental Baking Test (Blish, 1928). One-third of the remaining aliquot was spread out in a thin layer on a greased glass slab and frozen as in previous experiments. The remaining two-thirds of the aliquot was fermented an additional 2½ hours in an air thermostat at 30° C., and half of that in turn frozen. The remaining portion was fermented 2½ hours longer, or a total of 5 hours, and likewise frozen.

To the second dough an equivalent of 0.01% pepsin was added, or 10 mg. of pepsin to every 100 gm. of flour used. Scale pepsin was first dissolved in distilled water so that 10 cc. would contain 30 mg. pepsin. A 10 cc. portion of this pepsin solution was added to the dough before mixing and this water compensated for in the addition of the rest of the requisite water. This dough was aliquoted as in the instance of the control, thus baking two portions and fermenting and freezing the third.

A third dough was made up in the same manner as the control with the addition of an equivalent of 0.5% of Diamalt which had

been manufactured in such a manner as to avoid inactivating the malt enzymes. This dough was treated like the other two.

With this quantity of added enzymes no apparent change in the physical properties of the dough could be noted as fermentation progressed, except for the usual slackening that occurs in all doughs while ripening.

This dosage of pepsin did not seriously impair the bread-making qualities, as the following tabulation of the baking test data shows:

TABLE VII

BAKING TEST DATA OF CONTROL, PEPSIN, AND DIAMALT DOUGHS

	Loaf Volume	Color Score	Texture	Grain
Control	410 405	98y	99	99
Pepsin, 0.01%	455 450	98y	97	97
Diamalt 0.5%	430 400	96y	80	80

In the instance of the pepsin dough, an average increase of 45 cc. (about 10%) in loaf volume was experienced and the grain and texture were nearly equal to the control. The Diamalt dough had a poorer grain and texture, which rendered the crumb somewhat darker in color.

The frozen portions of these doughs were ground up and dehydrated with acetone as before. It was decided to limit the peptization studies of these doughs to one saline solution until larger discrepancies were encountered. K_2SO_4 solution was employed, as it is the one most commonly used in peptization work. The same concentration was used as before, namely, 0.5 M.

In the case of the control dough the same depression occurs in the amount of peptizable proteins at the 2½ hour fermentation interval as was pointed out in the instance of acetone-dehydrated doughs fermented 0, 2½, and 5 hours. A slight increase in this fraction was experienced in the other two doughs as fermentation progressed. An increase from 10.7% total protein peptized to 13.7% occurred in the case of the pepsin dough from the time the dough was first mixed until it had fermented 5 hours. In the Diamalt dough the increase in the amount of total protein peptized ranged from 14.4% in the freshly mixed dough to 18.0% in the 5 hour dough. These results appear in Table VIII and Figure 6 together with other data, which will be discussed later.

TABLE VIII

PERCENTAGES OF TOTAL PROTEIN EXTRACTED BY 0.5 M K_2SO_4 SOLUTION FROM A CONTROL DOUGH AND FROM DOUGHS TO WHICH ENZYMES WERE ADDED

Hours Fermentation	Control Dough	0.01% Pepsin Dough	0.02% Pepsin Dough	0.05% Diamalt Dough
0	14.98	10.68	10.69	14.45
2½	11.73	12.83	11.56	16.21
5	14.50	13.71	12.50	18.05

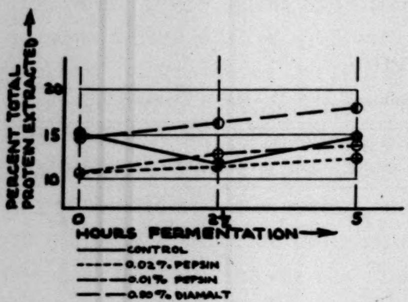


Figure 6. Percentages of total protein extracted by 0.5 M K_2SO_4 solution from a control dough and from doughs to which enzymes were added.

These data show that if there are any changes in the structure of the gluten proteins occasioned by the action of these added proteases during the time of fermentation of bread dough, this method of peptization is not capable of demonstrating them. It is conceivable that such proteases as pepsin and those found in Diamalt are not capable of cleaving the protein molecule into simple enough forms to be differentiated from the native proteins by such peptization. These experiments also indicated that the pepsin dosage used might not be sufficiently large to bring about any substantial change. These experiments were therefore repeated, using twice as much pepsin as in the first instance, or an equivalent of 0.02% pepsin.

The pepsin dough in this instance became more slack as fermentation progressed than did the 0.01% pepsin dough. In handling the doughs the operator was able to discern differences effected by this amount of added protease. The bread baked from this dough was not rendered inferior as to volume, grain, and texture by this treatment, as the baking test data show in Table IX. These doughs were re-baked on two different days and the same results obtained. As a supplementary study on the addition of proteases to bread doughs, doughs containing 0.035% pepsin and 0.02% trypsin were baked in order to determine the effect of larger quantities of proteases.

In all cases the bread containing proteases had a larger loaf volume than did the control. The grain and texture were also as good as the control, with the exception of the dough to which 0.035% of pepsin was added. It is probable that the smaller amounts of proteases (up to 0.02%) tend simply to soften the gluten and thus increase its elasticity toward expansion without affecting the qual-

ity of the gluten. Quantities of pepsin larger than 0.02% possibly have a weakening effect upon the gluten, thus hindering its gas-retaining capacity and in turn producing a poorer loaf of bread. The 0.02% pepsin dough was subjected to the same K_2SO_4 peptization as the previous doughs. These data are included in Table IX.

TABLE IX

Baking Test Data of Bread Containing Added Proteases

	Loaf Volume	Texture	Grain
	cc.		
Control	430		
	440	99	98
Pepsin 0.01%	490		
	495	99	99
Pepsin 0.02%	490		
	480	98	99
Pepsin 0.035%	465		
	465	90	90
Trypsin 0.02%	465		
	475	99	98

In the pepsin dough that had been freshly mixed 10.6% of the total protein was peptized by the K_2SO_4 solution. This value increased to only 12.5% in the dough that had fermented 5 hours. These data show that the two pepsin doughs, containing 0.01% and 0.02% pepsin, exhibited very nearly the same behavior, within the range of experimental error, toward this peptizing medium, even though the colloidal and physical properties of the dough were different. It is possible that these changes in colloidal properties are not accompanied by sufficient alteration of the chemical character and properties of the gluten proteins to be detected by this means.

To partially confirm this latter statement, the Sørenson formol titration was tried on the same material. The method used was that described by Cairns and Bailey (1927). A suspension of 12.5 gm. of dough in 100 cc. of toluene-water was agitated for 30 minutes in a mechanical shaker. The suspension was then whirled rapidly in a centrifuge for 10 minutes, and 50 cc. of the clear solution used for the titration. The solution was titrated to a distinct pink with phenolphthalein as the indicator. To this was added 10 cc. of a 40% formaldehyde solution (which had also been neutralized to phenolphthalein) and the solution again titrated with 0.1N NaOH. The results obtained were multiplied by 4 so as

to read in milligrams of amino nitrogen per 25 gm. of dough. The resulting data are recorded in Table X.

TABLE X

MILLIGRAMS OF AMINO NITROGEN PER 25 GRAMS OF DOUGH DETERMINED IN THE WATER EXTRACT BY SØRENSON'S FORMOL TITRATION METHOD

Hours Fermentation	Control	0.02% Pepsin	0.5% Diamalt
0	4.20	3.92	4.48
2½	1.64	1.64	2.24
5	1.64	1.64	2.80

These data show that there is but little nitrogen in the form of free amino acids even in doughs to which enzymes have been added. It is evident that this method is of little value in measuring proteolysis in bread doughs. Even though some free amino groups might be liberated during fermentation, the increase does not appear here. It will be noted that there is an actual decrease in free amino groups as fermentation progresses. The yeast present in the dough may have utilized these free amino acids in its metabolism, and at a rate exceeding their appearance in the dough in consequence of protease activity.

As pepsin does not carry proteolysis farther than the protease and, at the limit, the peptone stage, it seemed advisable to use an enzyme capable of hydrolyzing proteins to the amino-acid stage. If differences then occurred between the control and trypsin doughs as to the amount of protein peptized, one could conclude that this method is capable of measuring such a change, and also that no change paralleling this is taking place during the fermentation of an ordinary bread dough. Trypsin is an enzyme of this nature, so a dough containing an equivalent of 0.02% of this enzyme was mixed, along with a control, and frozen as before. These samples were extracted with distilled water and with 0.5 M K^2SO_4 solution. These results are given in Table XI and Figure 7. In the doughs that were extracted with distilled water the same increases in the amount of peptizable proteins were observed as fermentation progressed as in previous experiments. It is interesting to note, however, that the differences between the control and trypsin doughs, as to the amount of peptizable proteins, were relatively small. In the water extractions taken at the beginning of the fermentation period differences occurred between the control and trypsin doughs of less than 0.6% of total protein extracted, rising to only 1.87% and 3.62% in the 2½ and 5 hour doughs

respectively. The K_2SO_4 extractions yielded differences less than 0.9%, rising to only 1.44% in both the 2½ and 5 hour doughs. From these data it seems evident that this method is of little value in determining the possible degradation products that may be formed during the period of ordinary bread dough fermentation.

Precipitation of the Primary Proteins by Salts of Heavy Metals.—At this point it became necessary to try other methods directed toward a means of detecting such differences in the proteins as may occur during fermentation. The value of salts of certain of the heavy metals for precipitating protein-like materials has long been known. Olsen and Bailey (1925) modified Scherning's $SnCl_2$ method for precipitating the primary proteins. This method was also used by Cairns and Bailey (1927) in studying the proteoclastic activity of flour.

Samples, weighing 12.5 grams, of the control, pepsin, and Diamalt doughs were suspended in 100 cc. of toluene-water and shaken for 30 minutes in a mechanical shaker. To this was added 10 cc. of the stannous chloride reagent and a few drops of brom-cresol purple. A 5% solution of sodium hydroxide was added until the solution acquired a faint blue color. The solution, after being made up to 250 cc., was filtered. A few drops of HC1 was added to the filtrate, followed by a sufficient quantity of 5% NaOH to produce a clear solution. After refiltration 50 cc. aliquots were used for the nitrogen determination of the constitutents not precipitated by the tin reagent. It is of importance that the solution be neutralized carefully or difficulty will arise in filtration. When the first tint of blue is noticed the optimum point is reached. An excess of deficiency of as little as 2 or 3 drops of the neutralizing solution will cause much difficulty. This procedure was applied to control, pepsin, and Diamalt doughs fermented up to 5 hours, and the data are presented in Table XII and Figure 8.

In the doughs to which enzymes had been added more nitrogenous material escaped precipitation than in the instance of the control. The pepsin dough showed the greatest increase, as fermentation progressed, in the percentage of total protein not precipitated by the tin reagent, while the rates of increase in the control and Diamalt doughs were nearly equal and of small magnitude. It will be observed that the results here are expressed as percentages of the total protein present in the solution and not as percentages of total protein in the dough, as the non-protein nitrogen usually is expressed, thus making small differences as recorded here less significant.

TABLE XI

Percentage of Total Protein Extracted from Control and Trypsin Doughs with 0.5 M K_2SO_4 and Distilled Water

Hours Fermentation	Distilled water extractions		0.5 M K_2SO_4 extractions	
	Control	Trypsin	Control	Trypsin
0	16.35	16.90	8.46	9.28
2½	18.87	20.74	7.45	8.89
5	23.82	27.44	7.65	9.09

TABLE XII

Percentage of Total Protein Not Precipitated by Tin Reagent

Hours Fermentation	Control	Pepsin 0.01%	Diamalt 0.5%
0	11.6	15.2	15.7
5	12.7	19.7	17.4

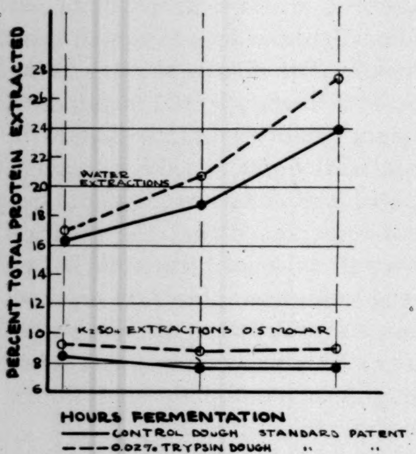


Figure 7. Percentages of total protein extracted from a control and trypsin dough with 0.5 M K_2SO_4 and distilled water.

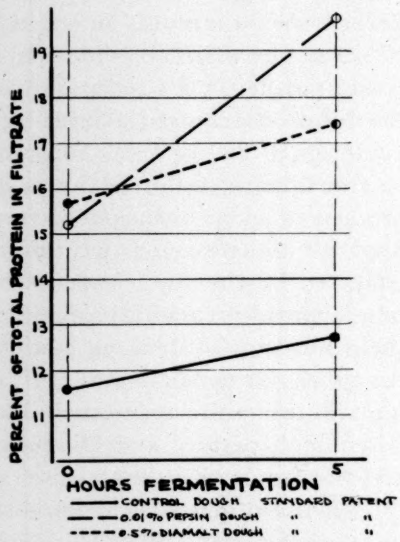


Figure 8. Percentage of total protein not precipitated by the tin reagent.

Blish (1918) has stated that some of the peptide nitrogen is not removed when the true proteins are precipitated with $CuSO_4$. This method described in his paper was tried, but the successive dilutions that must be made were so great that the sample contained too little nitrogen for accurate analysis. A similar method described by Cairns and Bailey (1927) as a modification of Ritthausen's method was used instead.

A 25 gm. sample of flour was shaken in 100 cc. of toluene-water for 30 minutes and 50 cc. of the centrifuged extract placed in a 100 cc. volumetric flask. To this was added 15 cc. of 0.5 N NaOH and enough 0.5 N $CuSO_4$ to change the color (in the presence of phenolphthalein) from a red through purple and blue to a green color. The addition of the correct amount of $CuSO_4$ is very important in this case in order to make possible a rapid filtration. A control and a 0.02% pepsin dough were used in this procedure and the results are given for the freshly-mixed dough and for a dough that had been fermented 5 hours. (Table XIII, Figure 9.)

TABLE XIII

PER CENT TOTAL PROTEIN IN AQUEOUS EXTRACTS OF AN ORDINARY DOUGH AND A DOUGH CONTAINING 0.02% PEPSIN NOT PRECIPITATED BY COPPER HYDROXIDE

Hours Fermentation	Control	0.02% Pepsin
0	4.2	3.2
5	5.2	8.5

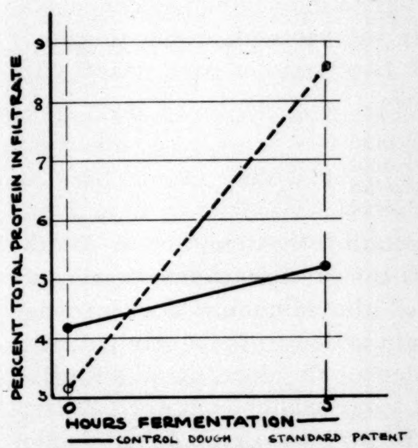


Figure 9. Percentage of total protein in aqueous extracts of an ordinary dough and a dough containing 0.02% pepsin not precipitated by copper hydroxide.

In the nitrogen determination the actual titration was multiplied by 8 to give the above results. Thus the small changes in the control dough could possibly fall within the range of experimental error, but it is obvious that the changes encountered in the pepsin dough are significant. The fact still remains, then, that there is no evidence of any substantial modification of the chemical structure of the gluten proteins in the fermentation of an ordinary bread dough. The amino nitrogen in the same extract was determined by the Sørenson titration method, and in no case were values obtained larger than 2.3 mg. of nitrogen per 25 gm. of dough. As before indicated, the amount of free amino nitrogen also diminished in this case with fermentation. From this it is evident that the nitrogen not precipitated by the copper is of a less complex nature than the true proteins but still in a form more complex than the free amino acids.

Determination of the Proteins Precipitated by Alcohol from a Water Extract.—When a sample of flour is extracted with water, and ethyl alcohol is added to the clear extract until a high enough concentration of the latter exists, some of the water-soluble material will precipitate. Because of the overlapping solubilities of the different proteins it is possible that these water extracts contain several of the wheat flour proteins, most probably albumin and leucosin. Bailey (1925) has stated that leucosin is soluble in pure water, and is relatively insoluble in aqueous alcohol solutions. Possibly the portion precipitated in this instance is mostly leucosin. In order to determine if any differences would appear in this fraction, an experiment was conducted in the following manner: A new set of doughs was mixed, one containing 0.02% pepsin, and a control dough. These were fermented, frozen, and dehydrated as before. To 10 gm. of each sample 100 cc. of toluene-water was added and the suspension shaken for 30 minutes in a mechanical shaker and centrifuged. The clear liquid was decanted into a liter beaker and the residue extracted again with 90 cc. of water and added to the first extraction. In order to determine the concentration of alcohol needed to precipitate the proteins in question, four aliquots were taken from each of two samples and mixed with alcohol in the following proportions:

(1) 75 cc. extract + 25 cc. ethyl alcohol
(2) 50 cc. extract + 25 cc. ethyl alcohol
(3) 25 cc. extract + 25 cc. ethyl alcohol
(4) 25 cc. extract + 50 cc. ethyl alcohol

In the first two instances no precipitation resulted, but in (3) the solution became quite turbid. In (4) the precipitation seemed quite complete. Thus, having determined the minimum concentration necessary, these solutions were again added to their original samples and by the addition of 95% alcohol the concentrations of all the samples were adjusted so that each beaker contained 2 parts of alcohol to one part of extract by volume, or 66 2/3% alcohol.

This mixture was allowed to stand overnight in a refrigerator at 5 ± °C., and filtered at the same temperature, the residue being washed several times with 66% alcohol previously cooled to

TABLE XIV

Mg. Nitrogen Precipitated by 66% Alcohol from Water Extracts of 10 gm. Dough

Hours fermentation	Control	Pepsin
0	3.2	3.6
2½	3.8	4.3
5	5.3	4.8

5±°C. The nitrogen in the residue and filter were determined by the usual Kjeldahl-Gunning method. A blank with the same filter paper was determined and subtracted from the reading. The data are recorded in Table XIV.

Although the precipitate was fairly bulky the nitrogen content was very low, as the data indicate, and therefore does not lend much information to this study. The very slight increase in this fraction as fermentation progresses is no doubt due to the fact that more protein is present in the extract from the fermented dough and consequently more is precipitated by the alcohol. If the amount of nitrogenous material precipitated by this method were large enough to be significant this experiment would have been repeated, determining the nitrogen content of the original extract, but these results do not justify such an experiment. However, this experiment indicates that no substantial change in this fraction accompanies fermentation.

The Hausmann Method.—Although the Hausmann method is used most frequently for the determination of the nitrogen distribution in a pure protein, it has also been used for material that is not a pure protein. Blish (1916) used this method for determining the distribution of nitrogen in wheat flour. He pointed out that the addition of starch to the flour effected changes in the distribution of the nitrogen.

Two doughs were mixed: (1) a control and (2) a 0.02% trypsin dough, and frozen and dehydrated as before. The Hausmann method was then applied to these doughs as follows: A total nitrogen determination was made on the dehydrated dough and the number of milligrams of nitrogen in 10 gm. of dough was calculated. A 10 gm. sample was hydrolyzed in a small Kjeldahl flask for 24 hours with 75 cc. HCl (sp.gr. 1.125). The hydrolysate was then concentrated in a vacuum still until as much HCl as possible was removed. After adding water, ethyl alcohol, and excess $Ca(OH)_2$, the ammonia nitrogen was distilled into standard N/14 acid at a temperature of 45°-50° C. The residue in the flask was filtered and washed with hot water until no test for chlorides was obtained, and the nitrogen determined on the residue and filter.

At this point a slight variation in the Hausmann procedure was employed. Several determinations were either lost or seriously in error due to the difficulty encountered in determining the nitrogen in the phosphotungstic acid filtrate received in working with such material as dough. Thus a similar procedure to that used by

Gortner (1913) was employed, in which the total nitrogen was determined on aliquots from the humin filtrate. Gortner represented the total nitrogen in the sample as the sum of the nitrogen obtained from the ammonia fraction, the humin precipitate, and the humin filtrate. In this work this method was also used, as it was found difficult to take a sample of the original hydrolysate due to the large quantity of non-protein material present. The values for the nitrogen in the phosphotungstic acid filtrate were also obtained by subtracting the nitrogen in the bases from the total nitrogen of the humin filtrate. In this manner better results were obtained. The data appear in Table XV.

TABLE XV
NITROGEN DISTRIBUTION IN CONTROL AND 0.02% TRYPSIN DOUGHS

	Hours Fermentation	NH_3N %	Humin N %	Basic N %	Non-basic N %	Total
Control	0	19.0	8.8	7.6	64.9	100.3
Control	2½	17.8	10.0	9.5	62.3	99.6
Control	5	18.4	9.8	10.1	60.0	98.3
Trypsin	0	18.4	6.7	8.1	59.4	92.6
Trypsin	2½	18.2	7.8	9.3	63.4	98.7
Trypsin	5	17.8	3.9	9.8	61.5	93.0

The results presented here are not so accurate as are usually obtained by the Hausmann method when applied to a pure protein, but a bird's-eye view of the whole table shows quite definitely there is no substantial alteration of any of these fractions during the fermentation of either dough. It should not be expected that very significant differences would occur in the various fractions. It is improbable that proteolytic enzymes in dough, whether they are originally present in the dough ingredients or added separately, would bring about any changes or degradations in the protein molecule other than would be subsequently produced by the hydrolysis with the strong hydrochloric acid used.

The Viscometric Method.—Johnson, Herrington and Scott (1929), and Collatz (1922) have suggested the possibility of using the viscometric method for studying proteolysis in fermenting bread doughs. In their studies flour-water suspensions were used which were subjected to extended autolysis. With such a procedure, the viscometric method revealed changes taking place in the flour proteins.

In a bread dough yeast is added which may or may not supply proteases to increase proteolysis. Sharp and Gortner (1924) have

pointed out another effect of yeast upon the viscosity of fermenting doughs. The doughs to which no yeast was added did not show any changes in viscosity during 5 hours fermentation that were greater than the experimental error. A marked increase in viscosity, however, was observed in the instances of dough containing yeast. This increase they attribute to the imbibitional capacity of the gluten. In all cases a maximum viscosity was experienced between 2½ and 3 hours fermentation.

Two standard patent flour doughs were mixed, fermented varying lengths of time, and frozen as before. One of these was dehydrated with acetone and the other ground to a fine powder in a meat grinder. Viscosity determinations were then carried out in the following manner: A sample equivalent to 21 gm. of the original flour was weighed and placed in a mortar. By adding 100 cc. of water very slowly and with continued stirring this quantity was diluted to a thin paste. To this paste 900 cc. of water was added and allowed to stand 45 minutes with occasional shaking. The material was allowed to settle and the top liquid decanted and discarded. To the residue 500 cc. of water was added and allowed to stand for an additional 15 minutes. After decantation of this water the residue was made up to 100 cc. and the viscosity determined on the Wallace and Tiernan Viscosimeter, after the addition of 0.5 cc. of 20% lactic acid. After a determination a portion of the material was removed and replaced by the same volume of water.

Similar determinations were made with equivalents of 18, 15, 12, and 9 gm. of flour per 100 cc. solution. In Table XVI the viscosities of the fermented doughs are indicated and are compared with the viscosity of the same flour. (Figures 10 and 11.) It will be noted that the viscosities of the doughs are less than those of the flour used in their preparation. The frozen dough in this instance gave larger viscosity readings as fermentation progressed up to 5 hours. With the acetone-dehydrated doughs the viscosity is greatest at the 2½ hour interval and falls off again with longer fermentation. This is in accordance with the findings of Sharp and Gortner in working with simple suspensions of fermented doughs.

The value of this viscometric method for studying small changes in the bread dough proteins is rather limited, since other factors, such as the effect of yeast on the imbibitional properties of the gluten proteins, tend to have a greater effect on the viscosity than the actual degradation of the proteins. Unless these other effects were standardized, it would be very difficult to definitely attribute any changes to protolysis. If yeast were simply omitted

from the dough formula for a reference basis of the imbibitional effect, one would not be dealing with the same conditions encountered in a bread dough.

In these experiments it has been noted that the final volume of the leached solution increased as fermentation progressed. After 5 hours of fermentation it was found difficult to reduce the residue to 100 cc. volume. In the instance of doughs which had been frozen

TABLE XVI

VISCOSITIES OF FROZEN AND DEHYDRATED DOUGHS AND THE ORIGINAL FLOUR

Grams in 100 cc.	Hours Fermentation	21	18	15	12	9
Flour	...	103	70	44	23	12
Frozen dough	0	10	8	5	2	..
Frozen dough	2½	23	15	11	6	3
Frozen dough	5	40	26	18	10	5
Flour	...	115	73	49	26	13
Dehydrated dough	0	27	20	13	9	5
Dehydrated dough	2½	59	37	23	13	6
Dehydrated dough	5	27	17	9	5	2

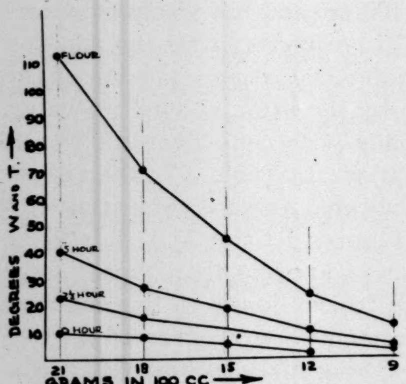


Figure 10. Viscosities of frozen doughs fermented 0, 2½, and 5 hours, and the viscosities of the same flour.

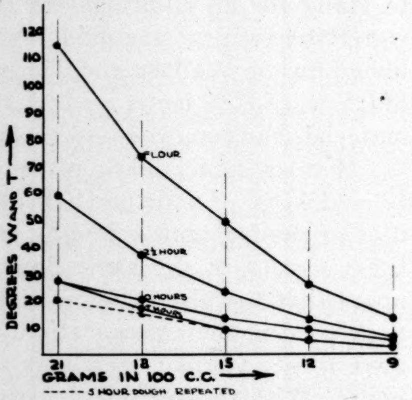


Figure 11. Viscosities of acetone-dehydrated doughs fermented O, 2½ and 5 hours and the viscosity of the same flour.

immediately after mixing, the gluten would not disperse on dilution to the same state of fineness as did the fermented dough gluten, but would tend to cohere and thus occupy smaller volume. These observations tend to suggest that the H-ion concentration may be the factor operating to produce such changes.

Doughs containing yeast and no yeast were mixed and prepared in the usual manner and the viscosities determined as

described above. In the yeast dough the viscosity reached a maximum at the 2½ hour interval and fell off again at the 5 hour interval. In the case of yeast-free dough, using the same procedure, it was very difficult to effect the dispersion of the gluten, and the resulting volume of the final residue was very small. The viscosity readings thus obtained from all stages of fermentation were below the range of dependability of the viscosimeter. The largest reading obtained was 4° W.&T., and no increase noted with fermentation greater than the probable error.

To partially establish the role of H-ion concentration in producing such differences in viscosity between yeast and no-yeast doughs, 3 doughs were mixed as follows: (1) ordinary yeast dough; (2) yeast-free dough; and (3) a yeast-free dough containing 2 cc., of 0.1 N lactic acid per 100. By this treatment the H-ion concentration of the third dough mentioned was brought to pH = 5.1. The viscosity determinations of these doughs are recorded in Table XVII and Figure 12.

TABLE XVII

Viscosity Changes of Dough Effected by Yeast and Lactic Acid Expressed in Degrees—Wallace and Tiernan

Grams in 100 cc.	Hours Fermentation	21	18	15	12	9
Yeast dough	0	28	20	13	8	4
Yeast dough	2½	43	29	18	10.5	5
Yeast dough	5	25	17	12	7	4
Yeast-free dough	0	5	3.5	2.5	..	..
Yeast-free dough	2½	3	2	..	..	..
Yeast-free dough	5	2	0	..	..	..
Yeast-free dough + 2 cc.	0	1	..	..	..	..
lactic acid	2½	22.5	17.0	12.0	8.4	4.0
	5	17.0	13.0	10.7	7.0	4.0

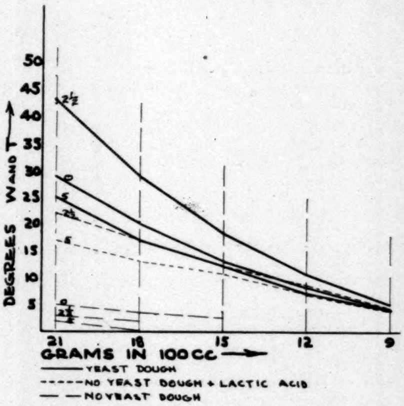


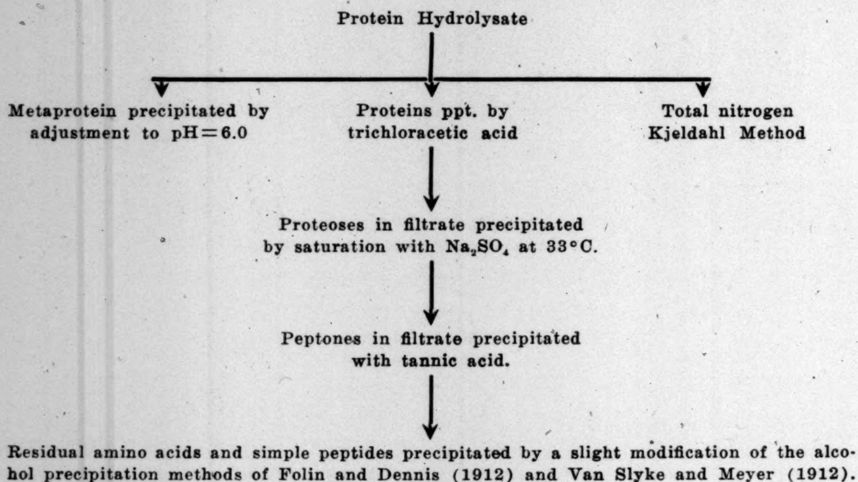
Figure 12. Viscosity changes of dough effected by yeast and lactic acid.

Again the yeast-free doughs yielded viscosity readings too low to be of much significance. The same general results were obtained with the yeast dough as heretofore. The acidulated yeast-free dough showed a maximum viscosity at the 2½ hour interval and the usual depression after 5 hours fermentation, thus comparing in general effect with the yeast dough. In this dough the gluten was also much more easily

dispersed after fermentation than before. This seems to indicate that the ease of dispersion, and possibly the viscosity, does not depend on the H-ion concentration of the dispersion medium, but on the contact of the gluten proteins with this acid medium during the period of fermentation. The fact that all the samples were brought to approximately the same H-ion concentration before the viscosity determinations by the addition of the strong lactic acid tends to substantiate the above statement that the length of the period of contact with this acid medium is the important factor.

In all probability the H-ion effect is not the only factor that is in operation in yeast-fermenting doughs to change the viscosity, but it may serve as an example, and until such effects are standardized this method will be of little value in studying proteolysis.

Wastenys' and Borsook's Method (1924) for the Fractional Analysis of Incomplete Protein Hydrolysates.—Wastenys and Borsook (1924) have been able to divide the constitutents of an enzy-



matic hydrolysate of protein into 6 fractions according to their relative complexity. The data so far presented in this paper indicate that some such method as this might be of importance in this study to differentiate the more complex protein split-products that may be derived by the action of the proteases present from the true protein material. A "flow sheet" of the method used by these workers is presented below.

For use in this study doughs from a high grade or patent flour and a low or clear grade flour were prepared. A sample of the

dough, equivalent to 25 gm. of flour, was extracted with 250 cc. of water by shaking for 60 minutes in a mechanical shaker. An attempt to concentrate the extract was made by distilling off some of the water at a temperature of 50°-55° C. under diminished pressure. Such manipulation effected the precipitation of some of the dissolved material. For this reason the extract was used in the original concentration. In order to express the results of each fraction as the percentage of total nitrogen in the extract, the total nitrogen was determined on the latter.

By adjusting the H-ion concentration of an aliquot of the original solution to pH 6.0, the meta-protein fraction is obtained. In the case of bread dough extracts, this treatment resulted in no visible precipitate, and the filtered solution contained the same amount of nitrogen as the original extract.

In precipitating the native proteins with trichloracetic acid, considerable difficulty was encountered. The addition of this acid in any quantity resulted in a very stable colloidal sol, which would not precipitate on standing or be removed by filtration. After numerous trials it was found that by the following procedure the precipitation could be effected: To 120 cc. of extract 30 cc. of 10% trichloracetic acid was added, thus yielding a solution containing 2% of this acid. In the instance of the standard patent doughs two drops of saturated NaCl was added at this point; this was not necessary, however, in the case of the clear flour doughs. After standing 1 hour the solution was boiled on a water bath for 30 minutes, using a reflux condenser to maintain the initial volume. The solution was then placed in the refrigerator maintained at 6°C., and left overnight. This procedure was very effective in precipitating the sol and leaving a clear solution. The solution was filtered and the nitrogen determined on duplicate aliquots. This procedure involves a modification of that used by Wastenys and Borsook and the fraction obtained may not represent the same complexity of protein material that they obtained by the use of this reagent. Hiller and Van Slyke (1922) have shown that percentages of trichloracetic acid as high as 5% have a slight hydrolysing effect on protein material. In these studies only 2% trichloracetic acid was used but the solution was boiled for 30 minutes. How much hydrolysis was effected by heating was not determined, but all samples were treated identically and thus comparisons can be made between samples, although this fraction may not represent the amount of native protein present. Wastenys and Borsook (1924) have indicated that the failure of trichloracetic acid to precipitate proteoses

is not very widely known. In their hands this reagent failed entirely to precipitate proteoses obtained from many widely-varying sources.

The remaining solution was then measured and boiled on a water bath for 3 hours to decompose the trichloracetic acid and drive off the resulting chloroform and carbon dioxide, after which the solution was made up to the original volume. Anhydrous sodium sulphate was added at this point until the solution was saturated. The excess of the salt was held as low as possible to avoid the error introduced by the removal of water by the hydrated crystals. The volume was again measured and a correction made for the increase in volume. The solution was then immersed in a large stirrer-equipped water thermostat, and kept at 33° ± 0.1°C. for 30 minutes. Although 32.5°C. is the temperature of maximum solubility, a temperature of 33°C. was maintained, since the transition point of this salt from the hydrated to the anhydrous form is 32.75°C. The small difference in solubility at these two temperatures is negligible, thus eliminating the error of the abstraction of water by the hydrated crystals. The filtration of this precipitated material at the same temperature (33°C.) was effected by placing a hot-water filter over the thermostat and water of constant temperature was circulated through the heating jacket. The receiving flask in this case was immersed in the bath itself. A definite volume of the filtrate was collected and duplicate nitrogen determinations made. The H-ion concentration of the solution in this procedure was not considered because such a "salting-out" would no doubt be independent of the H-ion concentration of the medium. In order to get the total amount in the original the results hereby obtained must be multiplied by the factor 11.5. This factor, being rather large, may tend to introduce some error, and thus the values here presented are not so accurate as might be desired, but would tend to bring to light any drastic changes that might be taking place. These data are presented in Table XVIII.

TABLE XVIII

Percent of Total N in Extract in the Form of "True Protein N" and "Proteose N."

	Hours Fermentation	True protein N %	Proteose N %
Standard patent dough	0	65.6	21.8
Standard patent dough	2½	64.8	21.8
Standard patent dough	5	65.0	24.3
Clear flour dough	0	61.9	21.3
Clear flour dough	2½	62.3	22.0
Clear flour dough	5	61.5	20.0

It will be noted that only very slight variations occurred between doughs fermented different lengths of time. The differences were no doubt within the range of experimental error. If the proteins were not broken down to the simpler stages, differences in these two fractions might be anticipated if any substantial changes were taking place.

The precipitation of the peptones in the Na_2SO_4 filtrate was carried out as described by Wastenys and Borsook, but the results obtained were erratic and of no value. In their work a pure protein was used and larger readings could be obtained throughout. With such material as a dough extract the nitrogen concentration is too low to carry out so many successive manipulations on a single sample. The readings obtained were so small that when they were multiplied by the factors involved by dilutions the results were quite useless.

Discussion

Considering all the data available, it would seem that the chemical structure or degree of aggregation of the gluten proteins is in no way altered during the fermentation of an ordinary bread dough, so far as can be detected by the methods employed in this study. The changes in the physical and colloidal properties of fer-...ing doughs, familiar to the experienced baker, cannot be due to an actual chemical change, but may be due to a simple dispersion of the colloidal complexes in which the protein molecules are left unaltered chemically.

The progressive increase in acidity as doughs ferment no doubt causes gradual changes in the dough, which are often attributed to proteolysis. This change in acidity may bring about changes in the imbibitional qualities of the gluten, thus effecting a possible swelling and syneresis of the colloidal gluten particles. Such alterations could readily account for the changes in viscosity as fermentation progresses.

The fact that such changes in the gluten proteins are taking place and still no parallel chemical changes can be detected tends to amplify the degree to which colloid chemistry comes into play in the making of bread.

Summary and Conclusions

Methods for the preparation of doughs for such studies are described.

(1) Fermentation of the doughs was inhibited at varying lengths of time up to 5 hours by freezing in a refrigerator at 17±°C. The frozen doughs were then ground to a fine powder for further manipulation.

(2) Doughs which had been frozen as in (1) were dehydrated with acetone, dried, and ground to the fineness of flour. This method proved very useful.

The experiments and results consist chiefly of the following:

(1) Comparisons were made between freshly mixed doughs prepared by the freezing method and the original flours as to the amount of total protein peptized by 3 saline solutions. Standard patent flour dough and the same flour behave almost identically on such treatment while clear flour dough and the same flour behave differently.

(2) Saline solution peptization of doughs fermented varying lengths of time indicated no change in the proteins as fermentation progressed.

(3) Distilled water and buffer solution peptization demonstrate the effect of H-ion concentration on the peptization of flour proteins.

(4) Acetone dehydration does not affect the proteins as the same results were obtained from acetone-dehydrated doughs as from frozen doughs.

(5) Addition of small quantities of proteases effects no change in peptization of dough proteins with salt solutions.

(6) Stannous chloride precipitations of the true proteins reveal no proteolysis during fermentation of an ordinary bread dough, but show slight cleavage of the gluten proteins in doughs to which Diamalt is added.

(7) Copper sulphate precipitates more protein material than stannous chloride but does not reveal any proteolysis during fermentation of an ordinary bread dough. Substantial changes in doughs to which pepsin was added were revealed by this method.

(8) Alcohol precipitation of the proteins in a water extract yields a precipitate low in nitrogen content and this fraction is not altered by fermentation.

(9) The nitrogen fractions separated by Hausmann's method are not altered by ordinary dough fermentation of 5 hours.

(10) Viscometric methods demonstrate changes in fermenting doughs but the method needs considerable standardization

before it can be of any use in studying proteolysis in bread doughs.

(11) A precipitation of the native proteins with trichloracetic acid and of the proteoses by sodium sulphate reveal no changes in the gluten proteins with fermentation.

(12) Fermentation does not affect the chemical structure of the gluten proteins so far as can be detected by any methods used in this study.

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WHEAT PROTEIN TEST DIGESTION STUDIES

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(Read at the Convention, May, 1930)

Although the protein test for wheat and wheat products has reached a high degree of refinement, it is not "fool-proof" and has its limitations which must be understood from the taking of the sample to the interpretation of the result. The relationship of the laboratory equipment and the testing method used are very important. The method adopted for regular commercial analysis should be that which is least sensitive to the varying and fluctuating factors encountered in making the test. Small variations in the amount of acid or sulphate measured out for each test, increased fume suction, decreased reflux action and a fluctuating heat source are factors affecting the concentration of the digest and their effect must be studied. In many laboratories where a high heat is available for protein digestion the Gunning method can be used to good advantage in terms of simplicity and economy; however, it is necessary for the chemist to know the limitations of the protein testing method he is using in order to know when unreliable results might make their appearance.

Coleman, Fellows and Dixon (1925) obtained data on protein digestion with the heat source adjusted to low, medium and high; viz., 50, 100 and 150 cc. of water evaporation from 200 cc. in 20 min.

Harrel and Lanning (1929) secured further information on protein digestion using a heat source a little above the high heat indicated by Coleman, Fellows and Dixon; viz., 180 cc. of water evaporation from 200 cc. in 20 min.

The following discussion and data is based on a still higher heat used for protein digestion; viz., 210 cc. $\pm$ 5 cc. of water evaporated from 300 cc. in 20 min. The digestion heat source for this work is a standard electric unit (110 V. Gilmer) such as is used in many laboratories, and this heat was attained by keeping the unit in continuous operation and the block thoroughly heated. A large number of heaters were tested and those of proper heat output selected for this work. The electrical input of these elements was approximately 640 watts.

Because Coleman, Fellows and Dixon concluded that heat was the most important factor in protein digestion it was thought that with this extra high heat output, a series of protein digestion

studies similar to the above mentioned studies might present a different picture of protein digestion. Although this digestion heat is greater than is normally used in some laboratories, information is given as to what can be expected when the heat source fluctuates to the extra high heat range.

All determinations made are based on a digestion mixture of 1 gm. sample, 20 cc. sulphuric acid, sp. gr. 1.84, using 0.5 gm. metallic mercury for the Kjeldahl method and 0.1 gram metallic copper for the Gunning method. Sulphate mixture (60% potassium sulphate and 40% sodium sulphate) was added according to the amounts specified for the various determinations.

Effect of Increasing Concentration of Sulphate in the Kjeldahl and Gunning Methods

It was first thought advisable to check the relation of an increasing concentration of sulphate between the Kjeldahl and Gunning methods, with a 40 min. digestion period. These results are shown for wheat and flour in Tables I and II and graphically in Figure 1.

These figures show that ammonia is lost in the Gunning method when the initial sulphate concentration is as high as 16 grams. It is to be noted that the ammonia loss is greater with the Gunning method than with the Kjeldahl method, as the sulphate concentration increases. The Gunning method with 13 gm. sulphate concentration is the procedure used regularly in thousands of protein determinations in this laboratory and has proved very satisfactory except in a few cases when fume suction was too great or the heater was abnormally hot.

Effect of Digestion Time and Extra High Heat

Sixteen grams of sulphate was not an excessive initial concentration for the Gunning method as was observed by Harrel and Lanning and it was thought that perhaps 40 min. was not the proper digestion time in these tests. A series of determinations were made, varying the time of digestion with extra high heat. These results are tabulated for wheat and flour in Tables IV and V. It is to be noted in Table IV that at no period of digestion can reliable results be obtained by the Gunning method with the higher concentration of sulphate; however, in Table V slightly low but fairly reliable results were obtained by the Kjeldahl method at this higher concentration.

Stability of Ammonium Sulphate in High Sulphate Concentrations and Extra High Heat

To obtain further information in regard to the low protein results secured in high sulphate concentrations, an artificial condition was produced in the digest by digesting 1.25 gm. sugar and 0.1250 gm. ammonium sulphate at varying initial sulphate concentrations for 40 min. The results are recorded in Table III, and represented graphically in Figure 1. It was found by experiment that with this amount of sugar in the digest the nitrogen lost, expressed in terms of per cent of protein, was similar to the nitrogen losses in the wheat and flour samples.

TABLE I—WHEAT

RELATIVE EFFECT OF INCREASING SULPHATE CONCENTRATION WITH COPPER AND MERCURY AND 40 MINUTES DIGESTION WITH EXTRA HIGH HEAT

Catalyst	Grams Sulphate	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average
Cu	7	13.78	13.74	13.74	13.78	13.78	13.74	13.76
Hg	7	13.82	13.89	13.85	13.86	13.78	13.86	13.84
Cu	10	13.84	13.88	13.84	13.80	13.84	13.76	13.83
Hg	10	13.88	13.88	13.88	13.80	13.84	13.84	13.85
Cu	13	13.88	13.84	13.80	13.84	13.84	13.80	13.83
Hg	13	13.84	13.88	13.84	13.84	13.84	13.80	13.84
Cu	16	13.16	13.08	13.40	13.24	13.32	13.20	13.23
Hg	16	13.76	13.84	13.76	13.80	13.76	13.80	13.79
Cu	19	8.60	10.68	13.24	8.16	11.96	11.40	10.67
Hg	19	13.28	12.60	13.36	12.80	13.56	12.56	13.03

TABLE II—FLOUR

Catalyst	Grams Sulphate	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average
Cu	7	12.02	11.98	11.98	11.94	11.98	11.98	11.96
Hg	7	12.06	12.06	12.02	12.02	12.02	12.06	12.04
Cu	10	12.08	12.04	12.04	12.04	12.04	12.08	12.05
Hg	10	12.08	12.04	12.04	12.04	12.08	12.08	12.06
Cu	13	12.04	12.00	12.08	12.04	12.04	12.04	12.04
Hg	13	12.04	12.00	12.04	12.08	12.04	12.08	12.05
Cu	16	11.92	11.48	10.76	10.96	11.96	11.96	11.51
Hg	16	11.96	12.00	12.04	11.92	12.00	12.08	12.00
Cu	19	10.76	7.60	7.38	8.44	9.84	10.88	9.15
Hg	19	11.28	11.40	11.64	11.68	12.04	11.96	11.66

TABLE III

0.1250 GM. AMMONIUM SULPHATE AND 1.2500 GM. SUGAR

Catalyst	Grams Sulphate	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Average
Cu	7	15.00	14.96	14.92	14.96	14.92	14.96	14.95
Hg	7	15.00	14.96	14.98	14.92	14.88	14.96	14.95
Cu	10	15.00	14.92	14.96	14.92	15.00	14.96	14.96
Hg	10	14.92	14.96	14.96	14.92	14.96	15.00	14.95
Cu	13	14.92	15.00	14.96	15.00	14.92	14.94	14.96
Hg	13	14.96	14.96	14.96	14.92	15.00	14.94	14.96
Cu	16	13.96	12.04	14.28	13.80	11.56	14.38	13.34
Hg	16	14.84	14.80	14.82	14.88	14.80	14.88	14.84
Cu	19	10.84	8.68	9.20	9.24	8.44	8.84	9.21
Hg	19	13.40	12.96	13.36	13.64	13.33	13.28	13.33

By increasing the sugar content of this digestion mixture the resulting ammonia loss appeared at lower initial sulphate concentration, and when the sugar content was decreased the loss appeared only at higher initial sulphate concentrations, the greater loss in all cases being with the Gunning method. This suggests

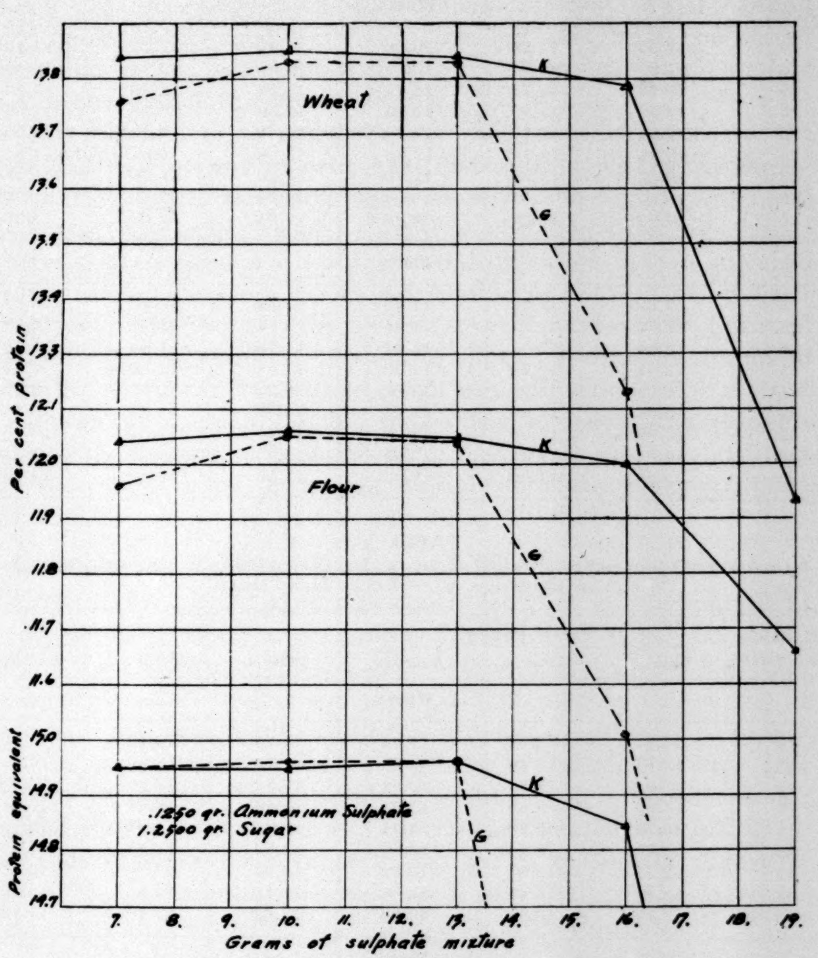

Fig. 1. Relative effect of increasing sulphate concentration with copper (G) and mercury (K) and 40 minutes digestion with extra high heat.

that 1.25 gm. sugar be used in blank protein determinations to produce foaming conditions and a resulting concentration similar to the actual test.

When 19 gm. of sulphate was used with this ammonia sugar sample in the Kjeldahl method the nitrogen loss occurred within

TABLE IV

GUNNING METHOD: EFFECT OF INCREASED SULPHATE CONCENTRATION AND TIME OF DIGESTION WITH EXTRA HIGH HEAT

Minutes Digested	13 Grams Sulphate			16 Grams Sulphate		
	Trial 1	Trial 2	Average	Trial 1	Trial 2	Trial 3
			Wheat			
10	13.32	13.28	13.30	12.72	13.12	11.16
15	13.52	13.64	13.58	11.92	12.04	12.16
20	13.72	13.76	13.74	13.24	11.88	11.26
25	13.80	13.80	13.80	11.80	13.78	11.92
30	13.86	13.74	13.80	11.86	11.00	13.00
35	13.84	13.88	13.86	13.00	13.64	11.80
40	13.82	13.78	13.80	13.16	13.00	11.48
45	13.84	13.84	13.84	11.00	13.72	11.36
50	13.88	13.92	13.90	12.94	12.04	13.36
			Flour			
10	11.72	11.72	11.72	11.32	10.72	11.04
15	12.00	11.92	11.96	10.44	11.12	10.80
20	12.04	12.04	12.04	11.24	11.64	11.28
25	12.04	12.00	12.02	11.68	11.56	9.44
30	12.04	11.96	12.00	10.22	12.00	12.00
35	12.08	12.00	12.04	11.68	10.64	10.16
40	12.00	12.00	12.00	11.20	10.88	10.80
45	12.02	11.98	12.00	11.92	11.40	9.52
50	12.08	11.96	12.02	11.52	11.78	11.72

TABLE V

KJELDAHL METHOD: EFFECT OF INCREASED SULPHATE CONCENTRATION AND TIME OF DIGESTION WITH EXTRA HIGH HEAT

Minutes Digestion	13 Grams Sulphate			16 Grams Sulphate		
	Trial 1	Trial 2	Average	Trial 1	Trial 2	Average
			Wheat			
10	13.64	13.64	13.64	13.76	13.76	13.76
15	13.84	13.76	13.80	13.76	13.80	13.78
20	13.92	13.80	13.86	13.72	13.80	13.76
25	13.82	13.86	13.84	13.80	13.88	13.84
30	13.88	13.80	13.84	13.92	13.80	13.86
35	13.80	13.86	13.83	13.82	13.82	13.82
40	13.80	13.84	13.82	13.84	13.76	13.80
45	13.88	13.84	13.86	13.76	13.80	13.78
50	13.88	13.88	13.88	13.82	13.70	13.76
			Flour			
10	12.00	11.96	11.98	11.84	11.92	11.88
15	12.00	12.00	12.00	11.96	11.96	11.96
20	12.04	12.04	12.04	12.04	12.00	12.02
25	12.00	12.00	12.00	11.90	12.00	11.95
30	12.08	12.00	12.04	11.96	12.00	11.98
35	12.08	12.00	12.04	12.02	11.92	11.97
40	11.96	12.04	12.00	12.00	11.92	11.96
45	12.04	11.96	12.00	11.92	11.92	11.92
50	12.04	12.00	12.02	11.96	11.92	11.94

the first ten minutes of digestion, or the period when the foaming was most evident. After this period no further losses were shown. Higher initial sulphate concentrations increase the foaming conditions both in this artificial digestion mixture and in the digesting of wheat and flour, the Gunning method showing this condition to a much greater extent than the Kjeldahl method. This foaming being more pronounced with the Gunning method is no doubt responsible for the lower, more erratic results given by this method in the higher sulphate concentrations.

It is reasonable to suppose that this foam structure in the digest does not permit an even heat distribution within the digestion mixture and a hot spot exists close to the heating element. This being true, better results should be obtained with high initial sulphate concentrations when the initial digestion heat is lowered or when the heat is more evenly distributed over the flask. Substituting for a distributed heat source several flasks were agitated constantly during the first 10 min. of digestion and the ammonia loss failed to appear at the end of 20 min. digestion. For a fixed heat source the Kjeldahl method shows less tendency toward foaming and this appears to be a factor in making it superior in these determinations, when excessive initial sulphate concentrations were used.

Comparisons Between Copper and Mercury as Catalysts

From Tables I and II it was concluded that approximately 13 gm. of sulphate was the maximum amount that could be safely used for the Gunning method and that it was also a very satisfactory concentration to use for the Kjeldahl method. A series of determinations were made, using this amount of sulphate to determine the comparative speed of digestion between mercury and copper as catalysts. These results are tabulated for flour, wheat, bran and shorts in Table VI and represented graphically in Fig. 2. From these figures it can be seen that the 35 to 40 min. digestion period with the Gunning method can be satisfactorily replaced by the Kjeldahl method, using a 20 to 25 min. digestion period.

These tabulations are in accordance with the observations of Harrel and Lanning that the weight of sulphate used in the digestion is a vital factor.

Conclusions

1. With extra high heat for protein digestion, precautions should be taken in increasing the initial sulphate concentration of the digest, in order to speed up the time of digestion, especially so with the Gunning method.

TABLE VI

RELATIVE SPEED OF DIGESTION BETWEEN COPPER AND MERCURY WITH EXTRA HIGH HEAT AND 13 GRAMS SULPHATE MIXTURE

Minutes Digested	Copper Catalyst			Mercury Catalyst		
	Trial 1	Trial 2	Average	Trial 1	Trial 2	Average
			Flour			
10	11.72	11.72	11.72	12.00	11.96	11.98
15	12.00	11.92	11.96	12.00	12.00	12.00
20	12.04	12.04	12.04	12.04	12.04	12.04
25	12.04	12.00	12.02	12.00	12.00	12.00
30	12.04	11.96	12.00	12.08	12.00	12.04
35	12.08	12.00	12.04	12.08	12.00	12.04
40	12.00	12.00	12.00	11.96	12.04	12.00
45	12.02	11.98	12.00	12.04	11.96	12.00
50	11.96	12.08	12.02	12.04	12.00	12.02
			Wheat			
10	13.32	13.28	13.30	13.64	13.64	13.64
15	13.52	13.64	13.58	13.84	13.76	13.80
20	13.72	13.76	13.74	13.92	13.80	13.86
25	13.80	13.80	13.80	13.82	13.86	13.84
30	13.86	13.76	13.81	13.88	13.80	13.84
35	13.84	13.88	13.86	13.80	13.86	13.83
40	13.82	13.78	13.80	13.80	13.84	13.82
45	13.84	13.84	13.84	13.88	13.84	13.86
50	13.88	13.92	13.90	13.88	13.88	13.88
			Bran			
10	16.09	16.09	16.09	16.53	16.79	16.66
15	16.35	16.47	16.41	16.79	16.84	16.81
20	16.66	16.79	16.72	16.88	16.84	16.86
25	16.66	16.82	16.74	16.88	16.90	16.89
30	16.88	16.88	16.88	16.88	16.88	16.88
35	16.92	16.86	16.89	16.88	16.92	16.90
40	16.88	16.84	16.86	16.88	16.88	16.88
45	16.84	16.88	16.86	16.88	16.88	16.88
50	16.93	16.84	16.88	16.92	16.80	16.86
			Shorts			
10	17.25	17.39	17.32	17.94	17.89	17.92
15	17.76	17.81	17.78	18.11	18.16	18.13
20	17.93	17.93	17.93	18.13	18.16	18.14
25	18.04	18.04	18.04	18.14	18.16	18.15
30	18.08	18.14	18.11	18.17	18.14	18.15
35	18.11	18.14	18.13	18.16	18.17	18.16
40	18.11	18.12	18.12	18.16	18.12	18.14
45	18.15	18.12	18.13	18.16	18.14	18.15
50	18.18	18.12	18.15	18.20	18.16	18.18

2. With extra high heat the Kjeldahl method gives a more stable condition for the ammonia residue than the Gunning method in high sulphate concentrations.
3. With extra high heat and approximately the highest safe initial

sulphate concentration protein digestion by the Kjeldahl method is 15 to 20 min. faster than by the Gunning method.

Acknowledgment

The author is indebted to Mr. M. C. Mann for his assistance and careful checking of the heating elements used in these tests.

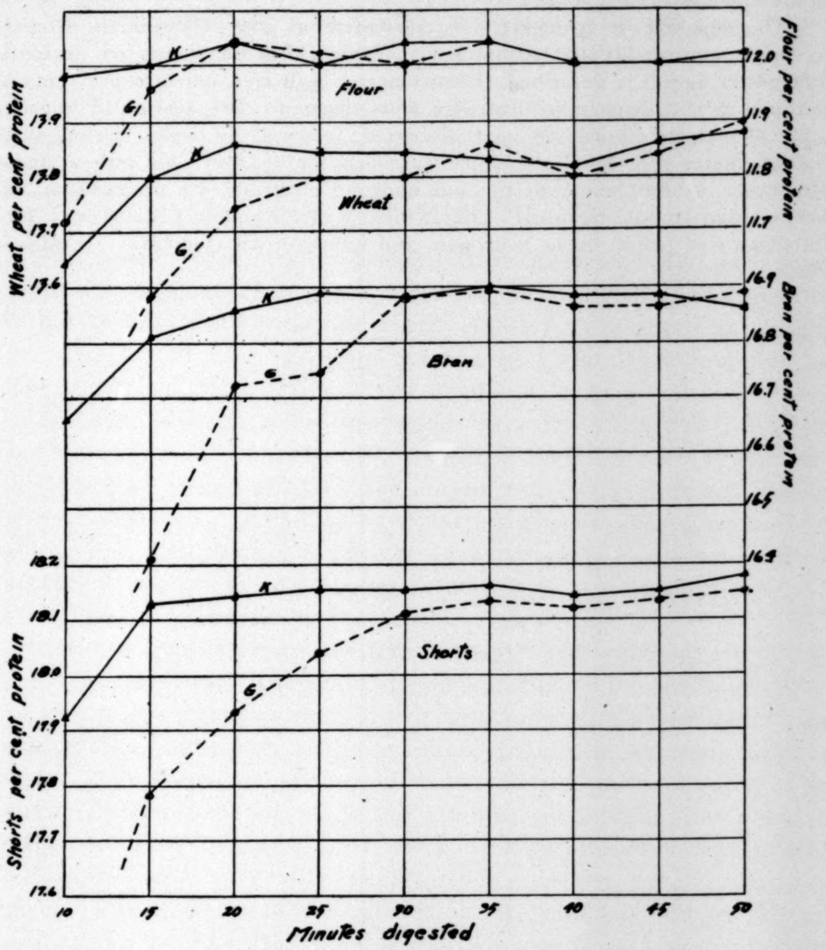

Fig. 2. Relative speed of digestion with copper (G) and mercury (K) with extra high heat and 13 grams sulphate mixture.

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